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Summary

Animal production in Ethiopia accounts for 15-17% of total Gross Domestic Product (GDP) and 35-49% of agricultural GDP. Unfortunately, the development and intensification of livestock productivity is hampered by devastating diseases, including African animal trypanosomosis. The most important trypanosomes, in terms of economic loss in domestic livestock, include: *Trypanosoma vivax*, *T. congolense* and *T. b. brucei*. Trypanosomes are often described as tsetse transmitted, but this is not always the case, with several species evolved for non-vector transmission or mechanical transmission by other flies. In Ethiopia, particularly the Northwest region is affected by both tsetse and non-tsetse transmitted *T. vivax*, with expected huge impact on livestock productivity. The control of trypanosomosis in Ethiopia relies on vector suppression and trypanocidal drugs, diminazene aceturate (DA) and isometamidium chloride (ISM) being the drugs most frequently employed. Consequently, drug resistance has become a common issue, especially for *T. congolense* in several areas. Although fragmented reports are available on the prevalence of the disease, attempts have not been done to demonstrate the relative importance of tsetse and non-tsetse transmitted *T. vivax* in terms of pathogenicity and drug resistance. This is important not only for evaluating treatment responses and to understanding host resistance/tolerance to the infections but also in the design of future vaccines and trypanocides, as well as the selection of hosts for tolerance or resistance to the parasites.

Therefore, our study started with a research questionnaire survey, a prevalence study and experimental studies were conducted to assess trypanocidal drug utilization practices, estimate prevalence of trypanosomosis and the consequent pathogenesis and drug resistance status of *T. vivax* isolates from tsetse-infested and non-tsetse infested areas of Northwest Ethiopia. The questionnaire survey showed trypanosomosis was a significant animal health constraint for 84% (n=84) and 100% (n=100) of the farmers questioned from non-tsetse and tsetse infested areas respectively. Responses on trypanocidal drug utilization indicated that risk factors for the development of drug resistance such as frequent treatment, poor handling and administration of trypanocides are prevalent and treatment failures are common. The investigation of bovine trypanosomosis carried out in the wet season of 2011 and 2012 showed the prevalence varied from 17.59% in 2011 to 25% in 2012 in tsetse infested areas, and the difference was ($P < 0.001$) significant. Similarly, in non-tsetse infested areas the prevalence varied from 3.85% in 2011 to 5.93% in 2012, but in this case the rise was not significant. *T. congolense* (75 %) was the most prevalent species followed by *T. vivax* (20.58%), and mixed infections (4.41%) in tsetse infested areas while in non-tsetse infested areas only *T. vivax* was detected.

For the investigation of pathogenicity and drug resistance against diminazene aceturate (DA) and isometamidium chloride (ISM), two consecutive experimental studies were performed on five different *T. vivax* isolates which we isolated from the field; three from a non-tsetse infested area and two from a tsetse infested area; in each case young Zebu cattle were experimentally infected with the isolates. Firstly one isolate from tsetse infested area (TT-ETBS1) and one isolate from non-tsetse infested area (NT-ETBD1) were tested. Secondly one isolate from tsetse infested area (TT-ETBS2) and two isolates from non-tsetse infested area (NT-ETBD2 and NT-

ETBD3) were tested. In total 94 young Zebu cattle of which 34 for pathogenicity and 60 for drug resistance studies were used to conduct four different experiments in two consecutive experiments.

Parameters measured for the investigation of pathogenicity included clinical, haematological, biochemical, cytokine, and gross and histopathological changes over the course of an infection. The result shows that both TT and NT *T. vivax* parasites caused clinical infection, with parasites appearing in circulation earlier in NT than TT infected cattle. The infections were characterized by reduced feed intake, intermittent pyrexia, undulating parasitaemia, rough hair coat, enlarged superficial lymph nodes, pale mucus membrane, lacrimation, emaciation and dehydration. Less frequently, diarrhea, mandibular oedema, nervous signs and recumbence were observed in both groups of infected animals. The mean packed cell volume (PCV), haemoglobin, total red blood cell and white blood cell counts were significantly lower and mean corpuscular volume (MCV) was higher in infected animals and consequently the type of anaemia observed was macrocytic normochromic in nature. The extent of changes in serum biochemical parameters, except for few cases of differences, infection with *T. vivax* derived from both tsetse and non-tsetse infested areas initiate similar biochemical changes indicative of significant pathology and there is no difference between parasite origins and the two experimental infections. Upon euthanasia, infected animals showed enlarged and haemorrhagic spleen; swollen and edematous lymph nodes; pneumonic and emphysematous lung; enlarged liver and haemorrhagic lesions on the brain and intestine. Histopathology revealed significant abnormalities characterized by lymphoid hyperplasia of the spleen, interstitial pneumonia, hepatic necrosis, tubulointerstitial nephritis, meningoencephalitis, and lymphoid hyperplasia in lymph nodes.

With respect to the cytokine analysis, significant increases in immune cytokine (IFN-γ, TNF-α, IL-12 and IL-10) secretions were demonstrated in the first experiment among groups infected by both tsetse and non-tsetse *T. vivax* isolates, as compared with the non-infected group, but no statistical difference was observed between the groups infected with the two parasite types. However, in the second experiment, tsetse adapted *T. vivax* elicited a stronger immunological response than the *T. vivax* from the mechanically transmitted population. Concomitant with this observation is that blood parasite load remained low throughout the experimental period in the group TT compared to the NT groups.

The drug resistance studies revealed that treatment of infected cattle with the recommend doses of trypanocides showed incomplete parasite clearance, consistent with the occurrence of resistant strains and adding to growing evidence that such resistance may be a problem. Furthermore at higher doses, one isolate from non-tsetse infested area (NT-ETBD2) was confirmed resistant and another one isolate (NT-ETBD3) was suspected for resistance to DA while one isolate from tsetse infersted area (TT-ETBS2) was suspected for resistance to ISM.

Generally, *T. vivax* from the two locations is almost equally pathogenic, parasite appearance in blood is faster and immune cytokine responses for the most part are lower in case of NT isolate whereas trypanocidal drug resistance is prevalent in both areas. Accordingly the following recommendations are forwarded; a) The present findings, reminded us that the impact of *T. vivax* should not be neglected in both tsetse and non-tsetse infested areas, b) Frequent administration, poor utilization and handling of trypanocidals could be sources of the problem for drug resistance and hence, appropriate corrective measures be taken in both tsetse and non-tsetse infested areas. In addition, further studies on the mechanisms of resistance for *T. vivax* is essential to monitor drug resistance and identify potential targets for new drugs, c) The early appearance of parasites in blood and lower cytokine responses in non-tsetse *T. vivax* infected groups needs further investigation from the parasite, vector and host angles.

Samenvatting

Dierlijke productie in Ethiopië maakt 15-17% van het totale BBP en 35-49% van het BBP van de landbouw uit. Helaas wordt de ontwikkeling en de intensivering van veeproductie belemmerd door verwoestende ziekten zoals Afrikaanse trypanosomose. De belangrijkste trypanosomen verantwoordelijk voor economische verliezen in veestapel omvatten *Trypanosoma vivax, T. congolense* en *T. b. brucei*. Trypanosomen staan erom bekend dat de tseetseevlieg als biologische verctor fungeert, maar dit is niet altijd het geval daar verschillende soorten zich verspreiden door transmissie met andere vliegen. In Ethiopië, met name de noordwestelijke regio, is er een grote impact op de productiviteit van dieren door zowel tseetseevlieg en niet-tseetseevlieg overgedragen *T. vivax*. De beheersing van trypanosomose in Ethiopië berust grotendeels op vector bestrijding en trypanocide medicijnen zoals diminazene aceturate (DA) en isometamidium chloride (ISM). Maar er wordt steeds vaker resistentie tegen geneesmiddelen gerapporteerd, met name voor *T. congolense*. Hoewel gefragmenteerde verslagen beschikbaar zijn voor de prevalentie van de ziekte, zijn er geen pogingen ondernomen om het relatieve belang van de tseetseevlieg en niet-tseetseevlieg overgedragen *T. vivax* qua pathogeniciteit en resistentie tegen geneesmiddelen aan te tonen. Dit is nochtans essentieel voor de evaluatie van behandelingen en het begrijpen van gastheer weerstand/tolerantie voor de infecties, maar ook in het ontwerpen van toekomstige vaccins en trypanociden, evenals de selectie van gastheren die tolerant of resistent zijn voor de parasieten.

Onze studie start met een enquête bestaande uit een vragenlijst. Dit als onderdeel van een studie naar de prevalentie en het effect van experimentele studies van trypanocide geneesmiddelen. Doel is een schatting van de prevalentie van trypanosomose in Noordwest Ethiopië en de daaruit voortvloeiende pathogenese en weerstand tegen trypanocide geneesmiddelen van *T. vivax* isolaten te bepalen. Dit zowel voor gevallen met tseetseevlieg als vector (cyclische) als andere vormen (mechanische) van transmissies. De vragenlijst wees uit dat 84% (niet-tseetseevlieg gebied) en 100% (tseetseevlieg gebied) van de boeren geconfronteerd werden met de negatieve effecten van trypanosomose. Ook werd duidelijk uit de vragenlijst dat risicofactoren voor het ontwikkelen van resistentie, verkeerd toegepaste behandeling, slechte behandeling en gebrekkige administratie gemeengoed zijn. Het onderzoek naar boviene trypanosomose gedurende het regenseizoen van 2011 en 2012 maakte duidelijk dat de prevalentie varieerde van 18% in 2011 tot 25% in 2012 in gebieden waar de tseetseevlieg aanwezig is. Deze toename is duidelijk significant ($P < 0.001$). Ook in niet-tseetsee aangetaste gebieden steeg de prevalentie van 4% in 2011 tot 6% in 2012, maar in dit geval was de toename niet significant. *T. congolense* (75%) was de meest voorkomende soort gevolgd door *T. vivax* (21%). Gemengde infecties (4%) kwamen enkel voor in tseetsee gebieden. In tseetsee vrije gebieden werd alleen *T. vivax* gedetecteerd.

Het onderzoek van de pathogeniciteit en resistentie tegen de trypanocide geneesmiddelen DA en ISM werden in twee fases uitgevoerd op vijf verschillende *T. vivax* isolaten die we uit het veld isoleerden; drie uit een tseetsee vrij gebied (NT) en twee uit een tseetsee besmet (TT) gebied. Telkens werden jonge Zeboe runderen experimenteel geïnfecteerd. In een eerste fase werden een isolaat van TT gebied (TT-ETBS1) en een van NT gebied (NT-ETBD1) getest. In een

tweede fase werden een andere isolaat van TT gebied (TT-ETBS-2) en twee andere van NT gebied (NT-ETBD2 en NT-ETBD3) getest. In totaal werden dus 94 Zeboes, 34 voor pathogeniteit en 60 voor resistentie studies gebruikt.

Het onderzoek analyseerde klinische, hematologische, biochemische, cytokine en histopathologische parameters. Zowel TT en NT *T. vivax* isolaten veroorzaakten een klinische infectie, maar parasitemie trad vroeger op in NT dan TT besmet vee. De infecties werden gekenmerkt door verminderde voederopname, intermitterende koorts, parasitemie die in ernst varieerde, ruwe vacht, vergrote lymfeklieren, bleke slijmvliezen, tranenvloei, vermagering en uitdroging. Minder vaak werden diarree, mandibulair oedeem, nerveus gedrag of langdurig neerliggen in beide groepen van besmette dieren opgemerkt. De gemiddelde hematocriet, hemoglobine, totale rode bloedcel en witte bloedcel waarden waren aanzienlijk lager dan in niet-besmette dieren en het gemiddelde rode bloedcel volume was hoger in besmette dieren. Dit alles duidt op een normochrome bloedarmoede. De omvang van de veranderingen van biochemische parameters in het serum zijn over het algemeen hetzelfde voor infecties met *T. vivax* uit gebieden met of zonder tseetseevlieg. Na euthanasie van de besmette dieren werd een gezwollen en hemorragische milt aangetroffen, de lymfklieren waren oedemateus en gezwollen, interstitiële pneumonie en hepatische necrose van hersenen en darm werden vastgesteld. Histopathologisch onderzoek toonde aanzienlijke afwijkingen aan gekenmerkt door lymfoïde hyperplasie van de milt, interstitiële pneumonie, hepatische necrose, tubulointerstitiële nefritis, meningitis, encephalitis en lymfoïde hyperplasie in lymfeknopen.

Wat de cytokine analyse betreft, werd in het eerste experiment een aanzienlijke stijging van cytokines (IFN-g, TNF-α, IL-12 en IL-10) aangetoond bij infecties met zowel *T. vivax* NT als TT isolaten. Deze waarden waren statistisch verschillend van de negatieve controlegroep maar niet verschillend tussen de twee geïnfecteerde groepen. In het tweede experiment echter, ontlokte het *T.vivax* TT isolaat een hevigere immunologische reactie dan de NT isolaten. Ook werd vastgesteld dat de parasitemie langer duurde tijdens de experimentele periode van de TT-groep dan de NT-groepen.

De resistentie studies toonden aan dat de behandeling van besmette dieren met de aangewezen dosissen van trypanocide geneesmiddelen tot een onvolledige verwijdering van parasieten uit het bloed resulteerde. Bij hoge doseringen bleek één NT isolaat (NT-ETBD2) resistent en een ander NT isolaat (NT-ETBD3) verdacht resistent tegen DA, terwijl één TT isolaat (TT-ETBS2) verdacht resistent leek tegen ISM. Samenvattend kunnen we stellen dat uit de bevindingen blijkt dat resistentie tegen geneesmiddelen een groot probleem in de bestudeerde gebieden vormt.

We kunnen besluiten dat *T. vivax* afkomstig van de twee locaties bijna eenzelfde pathogeniciteit vertonen. De parasitemie gebeurt vroeger en de immuun cytokine reacties zijn lager in het geval van 1 NT isolaat. Resistentie tegen trypanocide geneesmiddelen komt in beide gebieden voor. Aldus dienen de huidige bevindingen ons eraan te herinneren dat (a) de impact van *T. vivax* niet mag worden onderschat in beide gebieden, en dat falende administratie, verkeerd gebruik of foutieve samenstelling (onderdosis) van de trypanociden de bronnen van het resistentie probleem kunnen zijn en aldus corrigerende maatregelen moeten genomen worden.

List of Abbreviations

AAT	African animal trypanosomosis
AAU	Addis Ababa University
ALP	Alkaline phosphatase
ALT/GOT	Alanine amino transferase
AST/GPT	Aspartate amino transferase
CD	Curative dose
CI	Confidence interval
CVMA	College of Veterinary Medicine and Agriculture
DA	Diminazene aceturate
DIGIT	Drug incubation Glossina infectivity test
DIIT	Drug incubation infectivity test
DNA	Deoxyribonucleic acid
dpi	Days post-infection
ED	Effective dose
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organization of the United Nations
GALVmed	Global Alliance for Livestock in Veterinary Medicine
GGT	Gama-Glutamyl Transferase
GPI	Glycosyl Phosphatidyl Inositol
HCl	Hydrochloric acid
HE	Haematoxylin and Eosin
Hgb	Hemoglobin
IFN-γ	Interferon Gamma
Ig	Immunoglobulin
IgM	Immunoglobulin M
IL	Interleukin
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-1A	Interleukin-1alpha
IM	Intramuscular
ISM	Isometamidium chloride
IU	International unit
KUL	Catholic University of Leuven
LPS	Lipopolysaccharide
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MEP	Mitochondrial Electric Potential
MHC	Major histocompatibility complex
MPS	Mononuclear phagocytic system

NAHDIC	National Anima Health and Disease Investigation Center
NIC	Non-infected control
NO	Nitric Oxide
NT	*T. vivax* isolate infected group from non-tsetse infested area
NVI	National Veterinary Institute
OD	Optical density
PATTEC	Pan African tsetse and trypanosomosis eradication campaign
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
pi	Post-infection
PT	Post-treatment
RBC	Red Blood Cell
rpm	Revolutions per minute
RSMFP	Robert S McNamara Fellowships Program
SD	Standard deviation
SE	Standard error
SIRS	Systemic inflammatory response syndrome
SPSS	Statistical Product and Service Solutions
sVSG	Soluble variable surface glycoprotein
Th	T- helper cells
TLTF	Trypanosome released triggering factor
TNF-α	Tumor necrosis factor-alpha
TT	*T. vivax* isolate infected group from tsetse infested area
TT-ETBD1,2,3	Non-Tsetse area - Ethiopia Bahir Dar isolate 1, 2 and 3
TT-ETBS1,2	Tsetse area - Ethiopia Birsheleko isolate 1and 2 (tsetse area)
UoG	University of Glasgow
VAT	Variable antigen type
VLIR-UOS	The Flemish Inter University Development Cooperation
VSG	Variable surface glycoprotein
WB	World Bank
WBC	White Blood Cell
WTC	Wellcome Trust Center

Chapter I:

General Introduction on Animal Trypanosomosis

1.1. Animal Trypanosomosis

African animal trypanosomosis or Nagana is a disease caused by *Trypanosoma vivax, T. congolense and T. brucei* species. In domestic animals, these parasites cause a severe, often fatal disease while in wild animals the parasites cause relatively mild infections. As the illness progresses the animals weaken more and more and eventually become unfit for work, hence the name of the disease "Nagana" which is a Zulu word that means "powerless/useless" reviewed by Steverding (2008). The species of trypanosomes causing Nagana were discovered by different scientists in the beginning of the 20th century; Bruce (1895) discovered *T. brucei* as the cause of cattle trypanosomosis and in the meantime, the two other animal pathogenic trypanosome species *T. congolense* and *T. vivax* were discovered in 1904 and 1905, respectively by Broden and Ziemann (Steverding, 2008). *T. congolense*, *T. vivax* and *T. b. brucei* are the major causative agents of bovine trypanosomosis in sub-Saharan Africa (Duggan, 1977; Stephen, 1986). *Trypanosoma vivax* and *T. evansi* are also transmitted mechanically by biting insects, such as tabanids and stomoxys, in areas outside the tsetse belt as well as in South and Central America and Asia (Nantulya, 1990; Osório et al., 2008), while *T. equiperdum* is transmitted sexually and has a wider geographic distribution (Nantulya, 1990; Brun et al., 1998; Hagos, 2010).

Infections by these trypanosome species are not confined to cattle since they infect a wide range of domestic animals such as horses, camels, donkeys, mules, water buffalo, pigs, goats and dogs (Nantulya, 1990; Brun et al., 1998; Uilenberg et al., 1998; Stevens and Brisse, 2004). African trypanosomes also affect humans, causing Sleeping sickness or human African trypanosomosis. These parasites are *T. b. gambiense* found mainly in West Africa and *T. b. rhodesiense* located mainly in East Africa (Baltz et al., 1985; Stevens and Brisse, 2004). The South American trypanosome known as *T. cruzi* is transmitted by triatomas, and causes Chagas disease in humans (Cazzulo et al., 1997).

1.1.1. Classification of animal trypanosomes

The classification of trypanosomes has been based solely on medical and veterinary features. The unicellular trypanosome parasites belong to the order Kinetoplastida due to the presence of a kinetoplast at the base of the flagellum (Uilenberg, 1998; Stevens and Brisse, 2004). This kinetoplast contains the mitochondrial DNA of the parasite (Vickerman, 1985). The family Trypanosomatidae is subdivided into two genera: *Trypanosoma* and *Leishmania*, which are classified according to their morphology and range of hosts the parasite, infect (Momen, 2001).

The genus *Trypanosoma* is further subdivided into two sections; namely the Stercoraria and Salivaria, based on how the parasites are transmitted from the insect vector to the mammalian host once the parasite has completed its cyclic development (Uilenberg, 1998). In the Stercoraria section, the metacyclic trypanosomes develop in the hindgut and are transmitted via the faeces of the insect vector. The Salivarian parasites develop into the metacyclic stage in the anterior part of the digestive tract of the tsetse fly and they are inoculated via the saliva into the

mammalian host (Stevens and Brisse, 2004). The prominent feature of the Salivarian species is that they contain and express variable surface glycoproteins (VSGs); therefore, they are able to change their surface coats by a process known as antigenic variation (Donelson, 2003; Stevens and Brisse, 2004). Salivaria are further divided into four subgenera namely; *Duttonella*, *Nannomonas*, *Trypanozoon* and *Pycnomonas*. The *Duttonella* subgenera have a principal species known as *T. vivax* and a morphologically similar, but smaller species known as *T. uniforme*. *T. vivax* parasites mainly infect mammalian hosts in Africa and Latin America. The presence of a terminally situated large kinetoplast in *T. vivax* is the distinguishing feature that separates the *Duttonella* subgenera from the Salivarian trypanosomes (Stevens and Brisse, 2004).

1.1.2. Morphological characteristics of *Trypanosoma vivax*

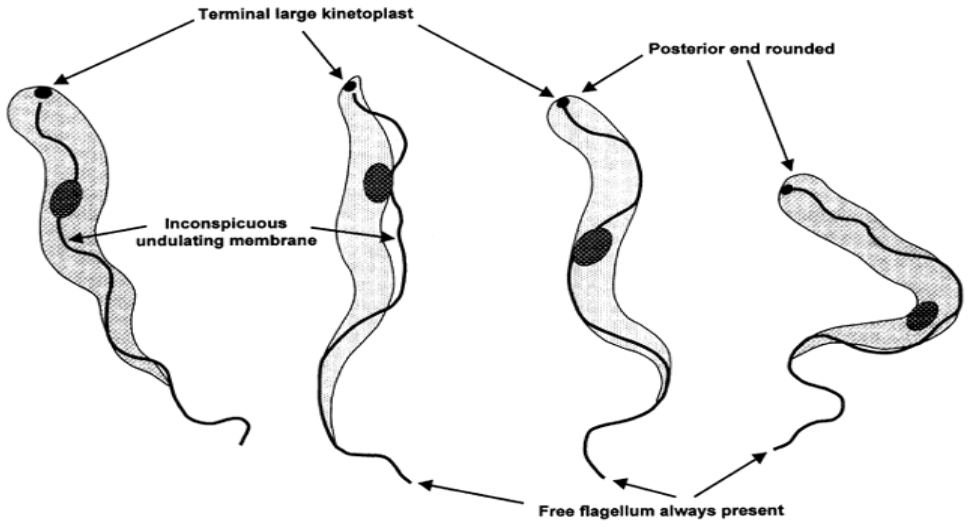

Figure 1.1. *Trypanosoma vivax* blood stream forms (Source: Uilenberg, 1998).

The increasing complexity of mammal-infecting trypanosomes has required their classification into subgenera, based on phylogenetic hypotheses. For example, for *T. vivax*, the subgenus *Duttonella* was introduced (Brener, 1979). The main characteristics of *Duttonella* bloodstream forms are large terminal kinetoplasts situated at a rounded posterior extremity, a medium developed undulating membrane, and a free flagellum (Figure 1.1). In Africa, bloodstream forms of *Duttonella* trypanosomes exhibit a certain degree of dimorphism. These include club-shaped forms with rounded bodies that are swollen posteriorly and taper abruptly toward the anterior end, and slender forms whose posterior end is also rounded, though not broader than the rest of the body, but tapers gradually toward the anterior end (Hoare, 1972). The undulating membrane exhibits a medium level of development (more than in *T. congolense* and less than in *T. brucei*), and a free flagellum is always present. One of the most distinctive traits of these trypanosomes is their large kinetoplast 1.1 μm in diameter (Hoare, 1972).

Biometric analysis of several samples of *T. vivax* showed that body length ranges from 18 µm to 31 µm (including the 3 - 6 µm long free flagellum), with mean lengths ranging from 21 µm to 25.4 µm (Hoare, 1972). Most trypanosomes of this species (90%) range from 20 µm to 26 µm in length. In fresh microscopic examinations, *T. vivax* is characterized by its capacity to quickly cross the microscope field of vision while other parasites move around. Its intermediate undulating membrane distinguishes it from large membrane ones exhibited by *Trypanozoon* parasites such as *T. evansi*. Among other characteristics, such as the size of the parasite, the round posterior extremity of *T. vivax* can also be distinguished from the very long, thin and rigid posterior end of *T. theileri* (Desquesnes, 2004).

1.1.3. The disease and its impact

Trypanosome infections take a variable course depending on factors associated with both the host and the parasites, but are characterized in most instances by the intermittent presence of parasites in the blood and intermittent fever. Three successive stages in infection may occur, namely acute, stabilization and chronic; however, death can occur at any stage. The initial stage is the acute phase of the disease, which may contain clinical signs such as, fever, anaemia, general loss of body condition, enlarged lymph nodes and spleen, weakness, lethargy, abortion and reduced milk production when trypanosomes invade and multiply in the blood stream of the infected animals (Stephen, 1986; Uilenberg, 1998). Death of the animal may occur in the first weeks or months of the infection due to the acute phase. About one or two weeks later, the sick animals usually have recurrent fevers for up to three months. Fever is the result of the contact between the trypanosomes multiplying in the host and the defense system of the host (Uilenberg, 1998).

If cattle survive the acute phase, infection tends to stabilize after six to eight weeks, characterized by stable PCV values and decline in the number of parasites in the blood, typical of the stabilization phase. The animal then enters the third or chronic phase of infection during which it may develop cachexia, intermittent parasitaemia and may become stunted, wasted and infertile. This chronic infection may lead to the death of the animal by congestive heart failure due to prolonged anaemia, damage to the heart muscles and increased vascular permeability (Uilenberg, 1998). In the chronic course, the disease lasting many months or even years is more common (Kettle, 1995). Infections with *T. b. brucei* and *T. b. evansi* can result in the invasion of the brain, eyes and skin, leading to nervous signs, discharges from the eyes and oedematous swellings under the skin (Kettle, 1995).

Tsetse-transmitted trypanosomosis is the main constraint to livestock production in the continent of Africa, preventing full use of land to feed the rapidly increasing human population (Shaw, 2004). It affects 37 sub-Saharan African countries, extending over 10 million km^2 of land (Erkelens et al., 2000). African animal trypanosomosis (AAT) is considered by many to be the single greatest health constraint to increased livestock production in sub-Saharan Africa with direct annual production losses in cattle estimated at US$ 0.6-1.2 billion. Estimates of the overall annual lost potential in livestock and crop production have been as high as US$ 4.75

billion. An estimated 45 to 60 million cattle and tens of millions of small ruminants are at risk from trypanosomosis (Gilbert et al., 2001). FAO (2000) estimates that about three million cattle die each year due to AAT. Furthermore, about 50 million people in Africa are exposed to the risk of contracting Sleeping sickness (Franco et al., 2014).

1.1.4. Life cycle and transmission

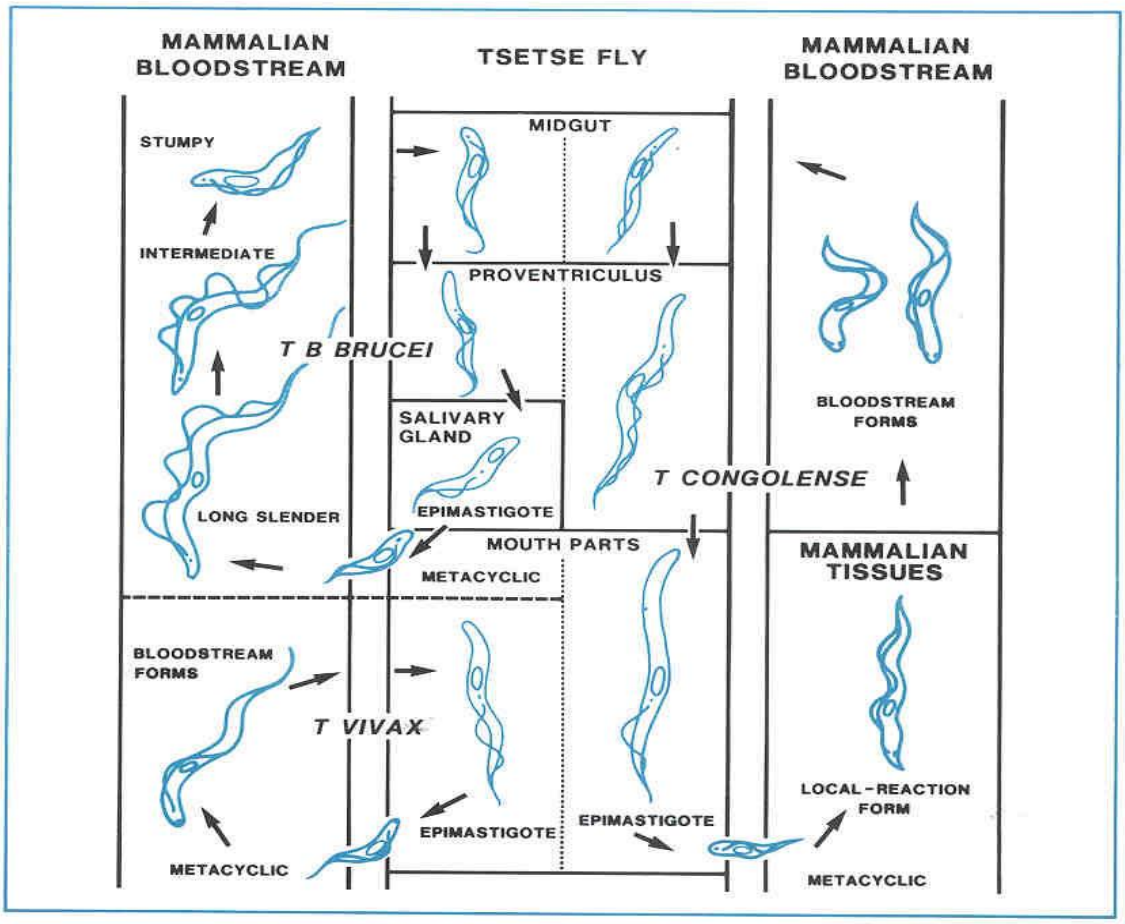

Figure 1.2. Life cycle of *T. b. brucei*, *T. congolense* and *T. vivax*. Heavy outlines indicate parasite forms with surface coats consisting of variable glycoprotein antigens. Light outlines indicate uncoated forms which are not infective to mammals. *T. b. brucei* develops in the tsetse midgut, proventriculus and salivary glands, where metacyclic forms occur which are infective to mammals. *T. congolense* develops in the tsetse midgut, proventriculus and mouth parts, where infective metacyclic forms are produced. *T. vivax* develops in the tsetse mouthparts (Source: ILRAD, 1982).

The lifecycle of the three main African trypanosome species are summarized in Figure 1.2 indicating the cyclical transmission: trypanosomes are transmitted through the bite of an infected tsetse fly. Tsetse flies get the infection when feeding on an infected animal; after implementation of the parasitic cycle in the fly (15-21 days) it becomes infective and may remain infective for the rest of its life. Transmission occurs in the early stage of the blood feeding, when the fly inject some saliva before sucking the blood of its host. Trypanosomes are

transmitted by both male and female tsetse flies (Uilenberg et al., 1998; Aksoy, 2003). However, *T. vivax* can also involved mechanical transmission by biting flies, especially tabanids and stomoxes, but possibly other biting insects (including tsetse flies) are the mechanical vectors. Mechanical transmission can occur when interrupted feeding is re-started on a new host; thus it is efficient inside a group of animals but has little chance to occur at distance. Trypanosomosis due to *T. vivax* has thus spread to some areas of Africa free or cleared of tsetse, and also in Central America and South America (Desquesnes and Dia, 2003). Others: vertical transmission can occur intra-utero and perorale transmission can also common for carnivore when eating fresh infected prey. Experimentally, most of the trypanosome species including *T. vivax* may also be transmitted by syringe passage of infective blood (Van den Bossche et al., 2000).

1.1.5. Development of immunity against trypanosomes

The outcome of disease occurrence depends on the interplay of the host, the agent (the parasite) and environmental factors. By adding or modifying the factors, the frequency of the occurrence of the disease can be changed (Thrusfield, 2007). Trypanosomes have evolved very sophisticated evasion mechanisms to survive in the chronically infected host. Well-documented evasion mechanisms include antigenic variation of the VSG and the induction of alterations in the host's defense system, such as excessive activation of the complement system leading to persistent hypocomplementemia, down regulation of nitric oxide production, polyclonal B-lymphocyte activation and marked immunosuppression (Guilliams et al., 2007). Most likely African trypanosomes induce other, yet undiscovered, changes in the physiology of the infected host, which might interfere with effective control of the parasite (Wei and Tabel, 2008).

1.1.5.1. Trypanosome antigens and exposure to host immunity

Trypanosomes causing morbidity and mortality in both man and livestock are unusual among the protozoan parasites in that they never enter the host's cells and yet persist for extended periods of time in mammalian blood and tissues. In the mammalian host, these parasites are completely surrounded by a dense immunogenic surface coat (12-15 nm thick) of a single polypeptide protein referred to as the variant surface glycoprotein (VSG) that shields invariant surface antigens from immune recognition. Moreover, trypanosomes constantly modify their VSG by the process of antigenic variation, resulting in the fluctuating waves of parasitaemia that characterizes African trypanosomosis (Borst, 2002; Pays, 2006).

Antigenic variation remains one of the most spectacular adaptive mechanisms exhibited by African trypanosomes and is the central most important immune escape mechanism by these parasites (Barry and McCulloch, 2001; Borst, 2002). Trypanosomes contain up to 1000 different genes in their genome which afford them extensive opportunities to escape host adaptive immune responses by displaying new coat antigens. The parasite has intrinsic mechanisms that ensure only one VSG gene is transcribed at any given time. By switching VSG genes and expressing a new variant antigenic type, trypanosomes evade B and T-cell mediated

immune responses. Furthermore, expression of VSG is central to the process of antigenic variation that eventually leads to exhaustion of the host immune system for the benefit of the trypanosome (Pays, 2006; Morrison et al., 2009).

VSG also has several effects on immune elements such as induction of autoantibodies and cytokines, in particular tumor necrosis factor (TNF)-α (Magez et al., 2008). According to Olsson et al. (1991) other trypanosome components and soluble factors, such as a trypanosome-released triggering factor (TLTF) which triggers interferon (IFN)-γ production by T cells, are also involved in modulation of the immune system by acting on the synthesis of immune elements. Elaboration of escape mechanisms to host immune defenses and induction of parasite growth factor production are well developed by trypanosomes. Because of the strong selection pressure from continuous contact with the host's immune system, the trypanosomes seem to have also developed several ways of evading immune killing through alteration of the host's natural and adaptive immune responses. For instance, *T. congolense* and *T. b. brucei* have been documented to induce a generalized state of immunosuppression following infection of cattle or mice (Namangala et al., 2000). The mechanisms of such immune depression seem to be mediated by both macrophages (Gomez-Rodriquez et al., 2009) and T cells (Namangala et al., 2000) with suppressive phenotypes. In the case of macrophages, the suppressive phenotype may be exhibited by both classically (caMFs) and alternatively activated cells (aaMFs) (Stijlemans et al., 2010). These macrophages subsets are antagonistically regulated, and their development is influenced by the cytokine environment. Whereas classically activated macrophages are induced by type I cytokines (TNF-α, IL-12, IFN-c) and inhibited by type II cytokines (IL-4, IL-10, IL-13), the reverse is true for alternatively activated macrophages (Kodelja et al., 1998).

1.1.5.2. Host responses to trypanosomes

The inoculation of trypanosomes into their mammalian hosts triggers a series of events involving, at first, innate immunity and, secondly, specific immunity. Cells of the macrophage lineage provide the first line of host defence against infectious diseases and, with dendritic cells, modulate downstream events that impact on the development of acquired immunity (Mansfield and Paulnock, 2005). The latter requires an efficient presentation of parasite antigens, activation of T and B cells implying specific antigen receptor recognition, and the development of effector cells and molecules. These mechanisms are regulated in a complex manner by multiple signals delivered through a large number of receptors transduced across the plasma membrane and processed. The production of antibodies to the VSG of *T. brucei* or *T. congolense* is the major early immune response. The first antibody to the VSG is of immunoglobulin M (IgM) class and is produced independently of T cells (Mansfield and Paulnock, 2005). Antibodies to the VSG are able to mediate control of the parasitaemia. During co-evolution with their hosts, trypanosomes have learned to cope with these host immune systems, by penetrating, diverting, and altering the numerous steps leading to the generation of an effective immune response. Major modifications of immune systems have been observed in trypanosomosis: lymphadenopathy, splenomegaly with destruction of lymphatic tissue architecture and hypergammaglobulinemia. However, their effectiveness is limited since most of the time;

parasites cannot be eliminated and prevailing immuno-pathological phenomena which induce tissue alterations (Philippe and Bernard, 2006).

Recent studies on the temporal transcriptional response of bovine peripheral blood mononuclear cells (PBMC) *in vivo* to a controlled trypanosome infection and identified time points indicated that there were increases in transcript levels for genes encoding pro-inflammatory mediators (IFN-γ, IL1A, TNF and IL12) in N'Dama by 14 day post infection compared with pre-infection levels. At the peak of parasitaemia a type 2 helper T cell (TH2) like cytokines were prevalent in the trypanosusceptible Boran cattle with increases in transcripts for the IL6 and IL10 genes in *T. congolense* infection (O'Gorman et al., 2006). Interferon-γ-induced NO production by macrophages from infected cattle was decreased and *in vitro* IFN-γ-induced synthesis of NO by peripheral blood mononuclear cells from uninfected cattle was suppressed by IL-10 (Tabel et al., 2000). Reports also indicated that whereas IFN-γ down regulated the synthesis of IL-10 in macrophages stimulated with bacterial lipopolysaccharide (LPS), IFN-γ up regulated the production of IL-10 in cultures of BALB/c macrophages pulsed with *T. congolense* (Kaushik et al., 2000). The findings of Tabel et al. (2000) indicate that the regulation of IL-10 synthesis by macrophages simultaneously stimulated by IFN-γ and *T. congolense* is different from the regulation of IL-10 synthesis upon combined stimulation with IFN-γ and LPS. The amount of IL-10 produced by macrophages was positively correlated with the amount of *T. congolense* homogenate used for stimulation (Tabel et al., 2000).

The effects of lipopolysaccharide (LPS) in inducing endotoxic shock in experimental animals have been studied extensively. Many cytokines are involved in endotoxic shock, such as IL-1, IL-12, TNF-α, IL-10 and IFN-γ (Billiau et al., 2001; Esche et al., 2001). The result of sepsis leading to a multiple organ dysfunction has been termed systemic inflammatory response syndrome (SIRS), associated with high serum levels of IL-6 and high IL-6/IL-10 ratio (Robertson and Coopersmith, 2006). Tabel et al. (2008) showed that susceptible BALB/c mice infected with *T. congolense* succumb to the infection due to the development of SIRS within 10 days of infection. This inflammatory syndrome is associated with hyper activated macrophages, an outburst of cytokine release, enlarged capillary bed, decreased blood pressure, drop of body temperature, hypomotility, and piloerection towards the terminal stage (Shi et al., 2003). SIRS also occurs in infected resistant C57BL/6 mice, when treated with antibodies blocking the IL-10 receptor (IL-10R). This early mortality is mediated by IFN-γ produced by MHC class II restricted CD4+ T cells. IL-10 appears to be crucial in controlling the lethal SIRS. It appears that IL-10 plays a dual role in African trypanosomosis: it exerts a detrimental role in mediating immunosuppression, but it is beneficial by controlling excessive lethal cytokine release by macrophages (Shi et al., 2003).

1.1.6. Pathogenesis

The precise pathogenesis of trypanosomosis remains far from clear. Four features: chancre, lymphadenopathy, anaemia, and tissue damages dominate the pathology of trypanosomosis.

1.1.6.1. Chancre

Infection becomes established at the site of inoculation of metacyclic trypanosomes in the skin, where a chancre may form. The chancre reaches a maximum diameter, of some 100 mm, ten to 14 days after an infective tsetse fly feed, its development preceding invasion of the bloodstream by trypanosomes, and is accompanied by enlargement of the draining lymph nodes which is known as lymphadenopathy. At this time the chancre begins to regress, and the characteristic series of intermittent parasitaemia begins. After an infection has become established, a protracted battle ensues as the parasite provokes an immune response.

1.1.6.2. Development of anaemia

The trypanosome species affecting man and domestic animals have been subdivided into two groups, the haematinic group (*T. congolense* and *T. vivax*) which remains in the plasma and the tissue invading group (*T. brucei, T. evansi, T. gambiense, T. rhodesiense* and *T. equiperdum*) found in extra and intra vascular spaces (Ngure et al., 2008). Because of their presence in the blood, these invading parasites produce numerous changes in the cellular and biochemical constituents of blood (Taiwo et al., 2003). Anaemia is a cardinal sign of trypanosomosis in many domestic animals, and the aetiology is probably similar in all species. There may be spontaneous recovery or death, but very often there is a chronic phase which is characterized by infrequent, low-grade parasitaemia. Animals lose weight and condition and, as a result of dyshaemopoiesis, remain anaemic. Despite the apparent absence of parasites in the circulation, red blood cell destruction continues, and insufficient erythropoietic compensation results in persistent anaemia.

The anaemia caused by animal trypanosomosis could be associated with decrease in PCV, haemoglobin and RBCs counts as reported by many authors in different animal species (Silva et al., 1999; Lukins, 1999) which may result from massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host. Reports also showed that anaemia with a significant reduction in PCV, total RBC count and hemoglobin concentration (Hgb) is a consistent finding in trypanosome infected cattle, goats, sheep, dogs and rabbits (Taiwo et al., 2003; Bisalla, 2007; Gow et al., 2007). On the other hand, significant increases were reported in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values, whereas no significant change was observed in mean corpuscular haemoglobin concentration (MCHC) (Nadia et al., 2012). Total white blood cell (WBC) counts were also varied from normal range to significant reduction (Taylor et al., 1996; Osman, 2012) in animal trypanosomosis. The mechanism or pathophysiology of anaemia in trypanosomosis is complex and multifactorial in origin (Naessens et al., 2005). Widespread phagocytosis of blood cells, such as erythrocytes and platelets, by macrophages invading bone marrow sinusoids is seen (Murray and Dexter, 1988). This anaemia could also be due to the haemolysins such as proteases, phospholipases and neuraminidases induced by the trypanosomes (Soulsby, 1982).

1.1.6.3. Tissue damage

The pathogenesis of tissue lesions varies with the species of trypanosome. *T. congolense* and *T. vivax* are mainly intravascular parasites; they induce changes in the endothelium of capillaries, and so indirectly cause damage to adjacent tissues. The severity of endothelial injury also depends on the interaction of host and parasite. Damage to endothelial cells by parasite products, immune complexes, vasoactive amines and cytokines increases vascular permeability. In *T. congolense* infections, a generalized dilatation of capillary beds, which alters the haemodynamics, is observed (Connor and Van Den Bossche, 2005). The concomitant anaemia and more sluggish tissue perfusion affect the exchange of metabolites and are associated with intracellular oedema of capillary endothelial cells. Fibrinous microthrombi form in response to endothelial damage. These changes can be prominent in *T. vivax* infections, with which disseminated intravascular coagulation is more commonly associated. Alterations to the microcirculation produce secondary degenerative changes in tissues. As capillary permeability increases, phagocytes and products of the parasite extravasate more readily and are responsible, in part, for some of the tissue lesions. An important feature of the pathogenesis of trypanosomosis is the effect on lymphoid tissue. As the disease progresses, the volume of tissue in the spleen, lymph nodes and bone marrow increases markedly. This hyperplasia of reticuloendothelial cells reduces lymphoid cell density, and eventual lymphoid depletion can occur.

Pathogenicity may differ within species of trypanosomes based on their geographical region. Some *T. vivax* trypanosomes from East Africa may cause an acute haemorrhagic disease in cattle, whereas *T. vivax* trypanosomes from West Africa may result in a milder non-haemorrhagic disease (Taylor and Authie, 2004). There has also been evidence of a correlation between the biological vector species and the level of virulence of *T. vivax* isolates. The *G. pallidipes* from central Kenya transmits a *T. vivax* isolate that causes acute disease and eventually leads to death in approximately one month in 70% of infected cattle. Conversely, the *G. fuscipes* transmits an isolate in Nyanza in the Southwest of Kenya, that causes a chronic infection and eventually leads to death in 100 to 160 days post infection (Osório et al., 2008).

1.1.6.4. Biochemical changes

Biochemical changes have been observed to be associated with trypanosome infection in animals and several factors have been found to influence the nature and severity of these changes. These include the species and strain of the parasite and host variability in susceptibility to infection. Biochemical evaluation of the body fluids gives an indication of the functional state of the various body organs (Anosa, 1988). Varying observations of biochemical changes have been reported in studies of trypanosomosis in animals (Awobode, 2006). The infection of cattle with *T. vivax* cause cellular and tissue damage, the changes being marked as the second parasitaemia build up. These changes seem to vary with the level of parasites in blood early in the disease, but as the disease progressed the changes displayed no particular pattern, thus

suggesting possible liver/kidney damage and the ineffective regulation of these biochemical parameters by the affected organs (Kadima et al., 2000).

1.1.6.4.1. Blood glucose

The only sugar found in blood is glucose, which is stored in the form of polymer glycogen. Normally, glycogen is found only in the intracellular form, whereas glucose is found almost exclusively in extracellular fluids. The level of glucose is maintained within a relatively narrow range and is controlled by several factors such as hepatic and renal uptake and release of glucose, glucose removal by the peripheral tissue, effects of hormonal influences on these processes, and intestinal absorption of glucose, which has only temporary effect on blood glucose levels (Coles, 1986). However, during a disease situation there is a decrease in glucose level in the blood. For instance, according to Kadima et al. (2000), serum glucose levels were significantly low on days 3, 4 and 5 post-infection of cattle with *T. vivax*, corresponding with the first parasitaemia build up, followed by a significant increase on days 7 and 8 post infection. This also coincided with disappearance of parasites from the blood. Subsequently, a gradual decrease from day 10 post-infection to below normal values was observed on days 11 to 13 post-infection. Opperdoes et al. (1986) indicated that the high parasitaemia observed coincided significantly with low levels of glucose.

Anosa (1988) also reported a significant decrease in glucose level in subclinical infection of camels with *T. evansi*. This situation could be explained by the parasites' need for glucose for their cellular metabolism through their glycolytic pathway. Increased metabolic rate caused by fever and hepatocytes degeneration could also be a reason for hypoglycemia in trypanosomosis (Cadioli et al., 2006). Hypoglycaemia in *T. congolense* and *T. brucei* infected animals from 14 days pi onwards were also reported on West African dwarf sheep. It was however, more pronounced in *T. brucei* infected animals on 14, 21 and 35 days pi (Taiwo et al., 2003).

1.1.6.4.2. Serum protein

The total plasma protein measurement which includes fibrinogen values may be affected by altered hepatic synthesis, rapid albumin breakdown or excretion during disease. There are no consistent reports on the situation of total protein during animal trypanosomosis; it could remain normal, increased or decreased (Anosa, 1988). Total proteins and gamma globulins increase while serum albumin decreases in several trypanosomes infection (Herrera et al., 2002). The increase in serum total protein may have been due to increased release of tissue specific enzymes and other intracellular proteins secondary to parasite induced cell membrane disruption (Orhue et al., 2005). It was also shown that an increase in total protein and globulins could be due to elevation in the gammaglobulin, as immunological response against the parasite (Hilali et al., 2006). Hypergammaglobulinaemia in African trypanosomosis on the other hand is usually associated with the increase in immunoglobin M (IgM) (Anosa, 1988). In contrast, a decrease in serum total protein concentration was reported in *T. congolense* infected sheep (Katunguka-Rwakishaya et al., 1995) that could be associated with sever reduction of albumin

level. For instance, the oedema reported in the different parts of the body during trypanosome infection could be due to a significant decrease in the albumin levels that possibly indicates liver damage (Orhue et al., 2005).

1.1.6.4.3. Serum lipids

Assay of triglycerides is one of the best method for diagnosing hyperlipemia, which is a syndrome characterized by negative energy balance and rapid mobilization of fatty acids derived from adipose tissue. The mobilized fatty acids ultimately result in fatty liver and subsequent hypertriglyceridemia (Forhead, 1994). A decrease in plasma levels of cholesterol and phospholipids have been reported in sheep infected with *T. congolense* and in man infected with *T. brucei*. These have been attributed to the fact that trypanosomes take up lipids for growth in the infected hosts (Katunga-Rawakishaya et al., 1995). Abnormalities of lipid metabolism have also been identified in several laboratory and domestic animals infected with various species of trypanosomes (Anosa, 1988; Adamu et al., 2008). Determination of total cholesterol, triglycerides and high and low density lipoproteins in serum showed that hypocholesteremia, low density and high density hypolipidemia on goats experimentally infected with *T. congolense* (Biryomumaisho et al., 2003).

1.1.6.4.4. Enzymes (AST, ALT and ALP)

Enzymes are protein catalysts synthesized by all living things. As catalysts their only biologic activity is to alter the rate at which equilibrium is established between reactants and their products. Serum (plasma) enzymes can be placed in to two distinct classes. The first consists of the plasma specific enzymes (e.g. cholinesterase, plasmin, thrombin, aspartate aminotransfera [AST], alanani aminotransferase [ALT] and alkaline phosphatase [ALP] often are liver synthesized enzymes) which are those that have a definite and specific function in plasma. Their normal site of action is in the plasma, and they are present in higher level in plasma than in most tissue cells. The second class consists of the non-plasma specific enzymes which are present in concentration much lower than their concentration in certain tissues. This group is divided into two subclasses: enzymes associated with cellular metabolism (metabolic enzymes) and enzymes of secretion (digestive enzymes). Enzymes of cellular metabolism are located within tissue cells and are present there in high concentrations as long as the cell remains healthy and the membrane is intact. The level of these enzymes in extracellular fluids and plasma is low. Secretory enzymes are rapidly disposed of through excretory channels such as the intestinal tract, urine, and bile and by inactivation and degradation their normal plasma levels are relatively low and are constant (Coles, 1986).

Reports on the increase in AST values during trypanosomosis on days 3 to 5 post infection could not have been due to any tissue damage, but rather due to parasites secreting it as part of their metabolites into blood circulation, since ALT and AST have been observed in homogenates and suspensions of trypanosome (Kadima et al., 2000). However, the increase following the second parasitaemic build-up suggests a possible progressive liver fibrosis

occurring as the disease progresses. In addition Abeer and Shaymaa (2011) reported that there is a significant increases in the activities of hepatic enzymes (ALT, AST ALP and GGT) which were observed as a result of hepatic damage which followed by hepatic necrosis. The causes of the elevation of AST levels in the serum of animals could also be necrosis of skeletal muscles and kidneys (Lording and Friend, 1991). Raised levels of ALP can be seen in inflammatory conditions of the gut and liver, while active hepatocellular damage is reflected by increases in plasma levels of AST and ALT (Lording and Friend, 1991). Significant increase in the serum activities of ALP, ALT, and AST were reported on single or mixed *T. congolense* and *T. b. brucei* experimental infection of mongrel dogs. These significant elevations indicated that the integrity of the vital organs like the liver in the case of elevations in ALP, ALT, and AST was compromised following infection with the trypanosomes. This might have led to the increased destruction of hepatocytes and other cells of the body like osteocytes, and skeletal muscle cells, giving rise to increased release of these substances in circulation (Ezeokonkwo et al., 2012).

1.1.7. Clinical signs

It is very common for one animal to be infected with not only more than one species of trypanosomes but also infected simultaneously with other blood parasites (Babesia spp., Theilria spp., Anaplasma spp. and Ehlrichia spp). This makes it difficult to conclude which clinical symptoms are attributed to a given parasite (Nyeko et al., 1990). Few adequately controlled studies have been made and thus a typical clinical response to any trypanosome is difficult to construct. The cardinal sign observed in the African animal trypanosomosis is anaemia. Within a week of infection with the haematic trypanosome (*T. congolense* and *T. vivax*) there is usually pronounced decrease in PCV, haemoglobin, red blood cells, and white blood cells levels and within two months these may drop to below 50 percent of their preinfecion value (Murray and Dexter, 1988).

Trypanosoma vivax has a variable incubation period, and, although it is considered to be less virulent for cattle than *T. congolense*, mortality rate of over 50% can occur. There seems to be a marked variation in the virulence of different strains of *T. vivax* but it remains the most important causes of the AAT of cattle, sheep and goat in West Africa. It causes mild disease in horse and chronic disease in dogs. Certain African isolates of *T. vivax* can cause acute disease accompanied by haemorrhagic syndrome (Mwongela et al., 1981; Roeder et al., 1984). Typical features of these infections include high, persistent parasitaemia, fever, pronounced anaemia and generalized visceral and mucosal haemorrhage particularly in the gastrointestinal tract. In the field the disease affecting adult cattle can be severe enough to lead to death or miscarriage even before diagnosis is reached and treatment can be started (Mwongela et al., 1981).

1.1.8. Diagnosis

The diagnosis of *Trypanosoma* infection is based on clinical signs and on the demonstration of the parasites by direct or indirect methods. The clinical signs of the AAT are indicative but are not sufficiently pathognomonic and diagnosis must be confirmed by laboratory methods. The

classical direct parasitological methods for the diagnosis of trypanosomosis, namely microscopic examination of blood or lymph node material, are not highly sensitive, but a number of techniques, including enrichment of the sample, rodent inoculation and molecular methods may increase the sensitivity (Murray et al., 1977; Solano et al., 2002). Indirect methods rely on serological tests by detecting specific antibodies developed by the host against the infection or, inversely, to demonstrate the occurrence of circulating parasitic antigens in the blood by the use of characterized specific antibodies. The detection of antibodies indicates that there has been infection, but as antibodies persist for some time (weeks, sometimes months) after all trypanosomes have disappeared from the organism (either by drug treatment or self-cure) a positive result is no proof of active infection. On the other hand, circulating trypanosomal antigens are eliminated quickly after the disappearance of the trypanosomes, and their presence therefore shows almost always that live trypanosomes are present in the animal (FAO, 2004).

Compared to standard parasitological techniques and serological methods, molecular diagnostic tools in particular the polymerase chain reaction, allow the detection of trypanosome infections with much lower parasite numbers, both in the vertebrate and in the insect host (Masiga et al., 1992; Desquesnes et al., 2001; Thumbi et al., 2010). The principle of molecular tests is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. A positive result indicates active infection with the trypanosome for which the sequences are specific, as parasite DNA will not persist for long in the host after all live parasites have been eliminated.

The diversity of PCR methods for identification of trypanosome taxa has also led to increased appreciation of trypanosome genetic diversity. In most cases, PCR diagnosis aims to identify the parasites at the species level, which can be done using various targets. The preferred targets are those which are present in a high copy number in the genome of trypanosomatids; the more copies of the target, the greater the chances of amplifying it by PCR. Single copy genes are more difficult to amplify (McLeod et al., 1997) and are rarely targeted since low parasitaemia is a characteristic of trypanosome infection and the sensitivity would be too low. Mini-chromosomes of the nuclear DNA contain satellite DNA which has been the most favoured target in the development of species-specific primers able to detect very small amounts of parasite DNA. Such primers were developed for the main pathogenic trypanosomes: *T. vivax* and *T. congolense* savannah, forest, and Kilifi types (Masiga et al., 1992). Mini-exon genes of the nuclear DNA are also multi-copy genes and have been a favoured target for the detection of *T. vivax* (Ventura et al., 2001). Other repetitive sequences have also been investigated, for example, in the detection of *T. vivax* DNA (Masake et al., 1997; Clausen et al., 1998; Morlais et al., 2001). Internal transcribed spacers (ITSs) of ribosomal DNA are a suitable target for PCR-based trypanosome diagnosis (Desquesnes et al., 2001); the use of primers developed from the ITS region would provide a multi-species-specific diagnosis using a single PCR. For identification of the trypanosome species in biological specimens from mammal or insect hosts, ribosomal genes and internal transcribed spacers are often chosen as target sequences (Desquesnes et al., 2001; Thumbi et al., 2010). For *T. vivax*, other target sequences have also

been used such as satellite and microsatellite sequences, spliced leader sequences, cathepsin L-like genes and proline racemase genes (Masiga et al., 1992; Ventura et al., 2001; Cortez et al., 2009; Nakayima et al., 2012; Fikru et al., 2014).

1.1.9. Control of trypanosomosis

Prevention and control of tsetse-transmitted trypanosomosis depends on methods directed to the vectors, the host and the parasites. Each of these approaches is useful but has important limitations, such as expense, environmental pollution and drug resistance.

1.1.9.1. Vector control

In the absence of vaccines and effective and affordable drugs, African trypanosomosis control relies heavily on vector control with eventual impacts ranging from reduction of fly populations to total eradication. Targets and traps have been effective in controlling populations locally and have been used extensively in agricultural settings and considerable success has been achieved by directly applying insecticides on animals (pour-on) (Rowlands et al., 2001). Discriminative spraying of just the resting sites of tsetse would reduce costs, cause less environmental pollution and would be easier to carry out as only a small percentage of the total tsetse habitat would be sprayed. However, the technique is labor intensive, demands high level of supervision and has effects on non-target organisms (Schofield and Kabayo, 2008).

An alternative approach that is area-wide in nature is the Sterile Insect Technique (SIT) which was applied in large scale tsetse eradication programs in some parts of Africa including Burkina Faso, Tanzania, and Zanzibar. The most notable example of the success of SIT, after tsetse population suppression with targets and pour-on, in Zanzibar where *G. austeni* has been eradicated from the Island (Vreysen et al., 2000). Despite such efforts and successes in vector control in tsetse infested areas of Africa, little attention has been paid to vectors of trypanosomes outside the tsetse zone such as for *T. vivax.*

1.1.9.2. Vaccination and use of trypanotolerant breeds

Enormous advances have been made in the area of immunology in the recent years, but in the case of protozoa in general, some of which ´inhabit the very arsenals of the immune response´ and trypanosomes in particular, many problems remain unsolved. The antigenic complexity of trypanosomes has thwarted attempts to develop a vaccine (Nantulya, 1986). Although potential immunological targets within the parasite have been identified, no vaccine will be commercially available in the near future. This conclusion, however, is based on the immunological knowledge of the blood stage of infection by African trypanosomes. Recently scientists found that intradermal infections with low numbers of trypanosomes is controlled by innate immunity and that this innate immunity is, in fact, compromised by suppressive adaptive immune responses. On the basis of these findings, they proposed a vaccine strategy that aims at a) preventing the early induction of suppression of Th1 responses by inhibiting the arginase

pathway, but enhancing the inducible nitric oxide synthase (iNOS) pathway of antigen-presenting cells and b) using the optimally lowest dose of antigens of the whole parasite to induce a Th1 imprint (Tabel et al., 2013). Furthermore, research into the biology of B cells has indicated that the problems might go further for the development of a functional vaccine such as the maintenance of high circulating anti-trypanosome antibody titres in the absence of parasite antigen might allow the immediate elimination of metacyclic parasite upon entry in the body, thereby avoiding the potential initiation of active B cell memory destruction by living and dividing parasites (Magaz et al., 2010).

The greatest hope for the immunological control of animal trypanosomosis lies in the exploitation of trypanotolerant breeds of livestock (Murray et al., 1990). Trypanotolerance is the ability to survive, reproduce and remain productive under trypanosomosis risk without the need of use of chemicals to control the vector or drugs to control the parasite (Murray and Trail, 1984). Contrary to the widespread belief that these trypanotolerant breeds are less productive than others because of their small size, it has been convincingly demonstrated that the productivity of trypanotolerant cattle is similar to or even better than that of trypanosusceptible breeds when kept under similar conditions (Murray, 1990). Because of the problems associated with existing control measures, it has been proposed that the use of trypanotolerant livestock represents at least a partial solution to the problem of livestock based agriculture in tsetse-affected areas of Africa. It has long been recognized that certain breeds of West African cattle are considerably more resistant to African trypanosomosis than others. This is especially true of the West African short-horned cattle (Muturu, Baoule, Laguna, Samba, and Dahomey) and the long-horned cattle (N'Dama). Trypanotolerance is an economically important trait of great biological interest. Understanding the function of the genes determining trypanotolerance in different models will contribute new insights in the control of parasite infection. Most of the research findings strongly support the inheritance of trypanotolerance is a multilocus model. Consequently more than 35 different quantitative trait loci (QTL) genes were identified with significant effects on trypanotolerance traits. Most of these QTL contribute to the three major trypanotolerance indicators: anaemia, body weight and parasitaemia controls.

1.1.9.3. Treatment

In sub-Saharan Africa, treatment and prophylaxis of trypanosomosis in cattle, sheep, and goats is dependent on the use of three compounds: diminazene an aromatic diamidine, homidium a phenanthridine and isometamidium a phenanthridine aromatic amidine. Quinapyramine a quinoline pyrimidine is recommended for use against trypanosomosis only in camels and horses (Peregrine et al., 1997).

Diminazene derivatives like diminazene aceturate have remarkable curative properties. It is very active, stable and easy to use and has very low toxicity. These advantages make it a practical and risk free trypanocides at least for cattle. It is prepared as a yellow powder and easily soluble in water. It is injected subcutaneously in cattle (slight local reactions possible) or intramuscularly (very rapid absorption) at a dose of 3.5 mg/kg live weight for treating *T. vivax*

and *T. congolense* infections. Infections due to *T. brucei* can be treated in horse and cattle with the dose of 7 mg/kg (Whiteside, 1962). Diminazene derivatives bind to DNA and interfere with parasite replication. This class of drugs has tendency to accumulate in tissue, therefore half-life is very long, which may lead to residual problems in food producing animals (Riviere and Popich, 2009).

Homidium salts are effective against *T. vivax* infections in cattle but less so against *T. congolense* and *T. brucei*. Their limited and protective activity in cattle depends on severity of challenge and may last three to five weeks. Homidium resistant trypanosome can be controlled by diminazene or isometamidium (Taylor et al., 2007). It is given to cattle in one or 2.5% solutions at the rate of 1 mg/kg. Novidium, which is a mixture of homidium chloride and bromide, has the same action as Ethidium. It can also be used in *T. brucei* infections in dogs at the rate of 3-5 mg/kg (Mira and Ralph, 1989).

Isometamidium is a phenanthridine aromatic amidine with a narrow therapeutic index which has been marketed for both a prophylactic and a therapeutic trypanocidal agent. Isometamidium chloride is used as curatively at lower dosage rates and prophylactically at higher dosage rates. It is usually prepared as red powder easily soluble in water. It is used in a one or two percent aqueous solution and administered by deep intramuscular injection at the rate of 0.25-0.5 mg/kg as curative, depending on drug resistance risk. Strain of trypanosomes resistant to isometamidium and other phenanthridine appear frequently, but they mostly remain susceptible to diminazene aceturate. It is given prophylactically to the animal at dose rate of 0.5-1 mg/kg and it gives protection for two to four months depending on the extent of infections risk. Dromedaries appear to be more sensitive to this drug than other animals (Mira and Ralph, 1989).

The concept of "sanative pair" treatment prescribes the use of a pair of trypanocides (e.g. Berenil and Homidium) which are chemically unrelated and, therefore, are unlikely to induce cross-resistance is a practical method in using trypanocidal drugs. One of the pair is used until resistant strains of trypanosomes appear and then the second is substituted and used until the resistant strains have disappeared from cattle and tsetse (Whiteside, 1962). However, some strains of trypanosomes have shown their ability to develop multiple drug resistance to chemically unrelated trypanocidals raising questions on the validity of "sanative pairs" (Mulugeta et al., 1997; Deluspaux et al., 2008)

1.1.10. Trypanocidal drug resistance

Drug resistance is the heritable loss of sensitivity of a micro-organism to a drug to which it was sensitive before. Trypanocidal drugs have been used in the field since 1960s, and resistance to each compound has been reported in trypanosome populations in a number of countries across Africa. Furthermore, multiple drug resistance has been reported, and in some instances is a particular threat to livestock production (Peregrine et al., 1997; Holmes et al., 2004). It is important to stress that drug resistance is not an "all or nothing" phenomenon and the degree of

drug sensitivity and resistance varies considerably between individual trypanosomes. Selection by drugs essentially takes place during asexual multiplication in the animal host, though there is some evidence that during passage through the tsetse fly, genetic exchange (sexual recombination) may occur at least in *T. brucei* (McDermott et al., 2003). In the past, the development of drug resistance in trypanosomes was mainly ascribed to their exposure to sub therapeutic concentration of trypanocidal drugs. Although this is an important aspect, the intensity of drug pressure (the treatment frequency and the degree of exposure of the parasite population) is probably even more important. The immunocompetance of the host also appears to play an important role in the development of drug resistance (McDermott et al., 2003).

1.1.10.1. Mechanisms of trypanocidal drug resistance

Trypanosome kinetoplast is the primary site of ISM accumulation and decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense* and indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes (1993) were also reported. Mulugeta et al. (1997) showed that the maximal uptake rates (V_{max}) of ISM in resistant *T. congolense* were significantly lower than in sensitive populations. It remains to be shown whether this is caused by a decreased number of protein transporters of ISM in the plasma membrane and/or by changes in the balance between influx and efflux. The role of nucleoside transporters in resistance to ISM by *T. congolense* remains to be examined, although changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei* (Ross and Barns, 1996). Changes in mitochondrial electrical potential have been demonstrated in ISM resistant *T. congolense* by Wilkes et al., (1997). Carter et al. (1995) showed that the accumulation of diminazene was markedly reduced in arsenical-resistant *T. b. brucei*, owing to alterations in the nucleoside transporter system. The mechanisms of resistance by trypanosomes to these drugs are vaguely unknown. However, there are indications that it is similar to that described for ISM (Peregrine et al., 1997).

Although diminazene probably exerts its action at the level of the kinetoplast DNA, this has not been proven *in vivo*, and other mechanisms of action cannot be excluded (Delespaux, 2004). Berger et al. (1993) showed that the accumulation of diminazene was markedly reduced in arsenical-resistant *T. brucei* owing to alterations in the nucleoside transporter system (P2). Increased resistance to diminazene was also observed in P2 deficient mutant of *T. brucei* (Matovu, 2003) and recently, RNA interference silencing the adenosine transporter-1 gene in *T. evansi* conferred resistance to diminazene aceturate (Witola et al., 2004). Those results are confirmed by De Koning et al. (2004) who conclude that the P2/TbAT1 gene mediates diminazene transport almost exclusively explaining the observed diminazene resistance phenotypes of TbAT1-null mutants and field isolates.

1.1.10.2. Detection of trypanocidal drug resistance

A standardized protocol for the assessment of susceptibility and resistance to trypanocidal drugs in mice or in ruminants has been described by Eisler et al. (2001) and Geerts et al. (2000).

The tests in ruminants consist of infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various dosages of trypanocides or following a simplified procedure with a single discriminatory dose as described by Eisler et al.(2001). The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation, and the curative dose (CD), i.e. the dose that provides a permanent cure (Sones et al., 1988). For these studies, the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of re-infection during the study. Blood from a group of infected cattle is inoculated into a single recipient calf, which is monitored, and later, when it is parasitaemic, treated with trypanocides at the recommended dose. A breakthrough infection, indicative of one of the inoculated trypanosome populations is drug resistant, is inoculated into groups of calves and mice to determine the level of drug resistance. This technique is useful in situations where laboratory facilities are very limited but it only allow a qualitative assessment and does not indicate how many of the isolates inoculated into a single calf were resistant. Further constraints to this technique are that not all populations might grow equally well and that sensitive isolates might overgrow resistant ones when inoculated together (Sones et al., 1989). A useful indication of the level of resistance can be obtained from studies in ruminants by recording the length of time between treatment and the detection of breakthrough populations of trypanosomes: the shorter the period, the greater the level of resistance (Ainanshe et al., 1992). The advantages of studies in ruminants are that most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field. The disadvantages are the long duration (a follow-up of 100 days is necessary to allow the detection of relapses) and the cost (purchase and maintenance of the animals are expensive). Furthermore, if only one isolate per animal is tested, it is usually impractical and too expensive to examine a large number of isolates.

Alternatively, after expansion of an isolate in a donor mouse, groups of 5 or 6 mice are inoculated with trypanosomes, 24 hours later or at the first peak of parasitaemia each group except the control group is treated with a range of drug doses. Thereafter, the mice should be monitored three times a week for 60 days. The ED50 or ED95 (the effective dose that gives temporary clearance of the parasites in 50 or 95 percent of the animals, respectively) can be calculated, as can the CD50 or CD95 (the curative dose that gives complete cure in 50 or 95 percent of animals, respectively). Sones et al. (1988) used groups of five mice, which allowed an easy calculation of ED80 and CD80 values (1 out of 5 mice was not cleared or cured). These figures should be compared with those obtained using reference sensitive trypanosome strains. The advantage of mouse assay is that it is cheaper than the test in cattle. There are several disadvantages, however: 1) most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice; 2) higher doses of drug must be used in mice (normally 10x higher) in order to obtain comparable results to those obtained in cattle because of the vast difference in metabolic size; 3) precise assessment of the degree of resistance needs a large number of mice per isolate, which makes it a labor intensive test identification of a discriminatory dose, and 4) it takes as long as 60 days to evaluate the drug sensitivity of an isolate (Sones et al., 1988).

In vitro tests using bloodstream or metacyclic trypanosomes can be used to detect resistance in *T. brucei* and *T. congolense* (Gray et al., 1993). A major disadvantage of these tests is the slow adaptation of the trypanosomes to the culture conditions. Furthermore, it is difficult to maintain *T. congolense in vitro* (Clausen et al., 2000). Two alternative approaches for *T. congolense* have been evaluated in which a short *in vitro* incubation in the presence of various drug concentrations is sufficient. The first approach is the drug incubation infectivity test (DIIT) where infectivity of the trypanosome is evaluated after incubation for 4 hrs in plasma samples derived from cattle treated with trypanocidal drugs. The second approach is the drug incubation Glossina infectivity test (DIGIT), and in this case the main limiting factor being the availability of tsetse flies (Knoppe et al., 2006).

The use of trypanocidal drug ELISA in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. The test is both sensitive, detecting subnanogram concentrations, and specific. It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma. One interesting finding has been that the drug disappears more rapidly in animals challenged and becoming infected with drug-resistant trypanosome isolates than in those challenged but protected against infection with sensitive trypanosomes (Eisler et al., 1994).
Resistance to ISM can be assessed under natural *Trypanosoma* challenge in the field using the 'block treatment' approach (Eisler et al., 2000). Two groups of infected cattle, either treated with 1 mg/kg ISM or untreated (each group consisting of 30 to 80 animals) are exposed to natural challenge and tested for the presence of trypanosomes in the blood using the phase contrast buffy coat technique every two weeks for two to three months. If >25% of ISM treated cattle become infected within eight weeks of exposure, drug resistance is strongly suspected. This approach can also be used for assessing whether there is suspected resistance to DA by treating the control group at the start of the experiment, and all animals that become infected during the trial, with DA and checking for the presence of parasites two weeks after treatment (McDermott et al., 2003). Furthermore, longitudinal parasitological field data can be suitably analyzed using appropriate statistical techniques to detect problems of resistance to DA (Rowlands et al., 1993).

Due to the vast limitations of the above listed techniques to validate drug resistance in trypanosomes, an alternative approach may be made to identify genetic markers for drug resistance, which might be developed into reagents for the identification of resistant trypanosomes using the polymerase chain reaction (PCR). A PCR-based test could provide a rapid and convenient tool, suitable for large-scale epidemiological surveys of livestock. Developments of such tests require the identification of genetic mutations that may be associated with drug resistance in livestock-infective trypanosomes (Vitouley et al., 2011).

1.1.10.3. Distribution of trypanocidal drug resistance

The first case of drug resistance in trypanosomes was reported in northern Nigeria (Naisa, 1967). At present, there are twenty-one African countries in which trypanocidal drug resistance

has been reported (Delespaux et al., 2008; Chitanga et al., 2011). The different species of trypanosomes and detection of resistance against trypanocidal drugs are summarized in Table 1.1. In addition, the occurrence of multiple drug resistance to diminazene, isometamidium and homidium has been reported in trypanosome populations in ten African countries (Delespaux et al., 2008). This is more worrying and threatening the last stand to overcome drug resistance through the use of the sanative pair (Mulugeta et al., 1997).

Table 1.1. Summary on drug resistant trypanosomes in different part of the world.

Country	**Trypanosome species**	**Resistant to (*)**	**References**
Burkina Faso	Tc	I	Pinder and Authié, 1984
		I,D,H	Clausen et al.,1992
	Tv	I,D	McDermott et al., 2003; Sow et al., 2012
Mali	Tc	I,D	Mungube et al., 2012
	Tv	I	Mungube et al., 2012
Mozambique	Tc	I,D	Jamal et al., 2005
Kenya	Tc	I	Gray et al., 1993
	Tc	I,D,Q	Peregrine,1997
Zambia	Tc	I,D	Chitanga et al., 2011
Zimbabwe	Tc	I,D	Joshua et al.,1995
Kenya/Somalia	Tv	I	Schönefeld et al., 1987
		H	Ainanshe et al., 1992
Nigeria	Tv	D, H, I	Ilemobade, 1979
	Tb	D, I	Kalu, 1995
Sudan	Tc, Tv, Tb	H	Abdel Gadir et al., 1981
Uganda	Tb	D, I	Matovu et al., 1997
Ethiopia	Tc	D,H,I	Mulugeta etal.,1997
	Tc	I,D	Afework et al., 2000
	Tc, Tb	I	Tewolde et al., 2004
	Tc	I, D	Shimelis et al., 2008
	Tv	I,D	Desalegn et al., 2010
	Tc	D,I	Moti et al., 2012
South America	Tv	D	Jones and Davila, 2001
China	Te	Q	Liao and Shen, 2010

(*) D = Diminazene aceturate, H = Homidium bromide (Ethidium), I = Isometamidium chloride; Q = Quinapyramine, Tc = *T. congolense*, Tv = *T. vivax*, Tb = *T. brucei*, Te=*T. b. evansi* (Source: adapted from Delespaux et al., 2008).

1.2. Trypanosomosis in Ethiopia

Ethiopia has the largest livestock population in Africa with 53.99 million cattle, 25.49 million sheep, 24.06 million goats, 1.91 million horses, 0.35 million mules, 6.75 million donkeys and 0.92 million camels (CSA, 2013). Livestock is a significant contributor to economic and social development in Ethiopia at the household and national level. Livestock accounts for 15-17% of total GDP and 35-49% of agricultural GDP. Unfortunately, the development and intensification of livestock productivity in Ethiopia is hampered among others by cross-border epizootic diseases such as African animal trypanosomosis (AAT). The most important trypanosomes, in terms of economic loss in domestic livestock include: *T. congolense, T. vivax, T. b. brucei, T. equiperdum* and *T. evansi.* The closely related *T. brucei* subspecies, *T. b. rhodesiense* causes human sleeping sickness was also reported (Abebe, 2005) as indicated in Figure 1.3.

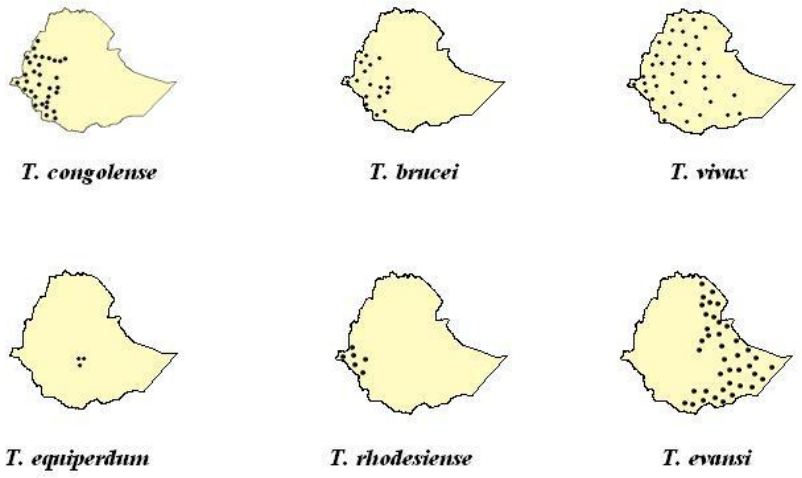

Figure 1.3. Distribution of pathogenic trypanosomes in Ethiopia (Source: Abebe, 2005).

1.2.1. Status of bovine trypanosomosis in Ethiopia

Most of the studies conducted in Ethiopia on trypanosomosis focused on tsetse transmitted trypanosomosis to determine the prevalence and impact of the disease (Codjia et al., 1993; Afewerk et al., 2000; Abebe, 2005; Dagnachew et al., 2005; Cherent et al., 2006; Fikru et al., 2012). In the Northwest region both tsetse and non-tsetse transmitted trypanosomosis (Abebe and Jobre, 1996; Cherenet et al., 2006; Sinshaw et al., 2006) are prevalent. In tsetse-infested areas of Ethiopia, *T. congolense* and *T. vivax* are the dominant species. Abebe and Jobre (1996) reported an infection rate of 58.5% for *T. congolense,* 31.2% for *T. vivax* and 3.5% for *T. brucei* in southwest Ethiopia. In the same report 8.7% prevalence rate was recorded in the highlands (tsetse free areas) of which 99% is due to *T. vivax.* The prevalence of bovine trypanosomosis in tsetse infested areas of Northwest Ethiopia was 17.07% (Dagnachew et al., 2005). Apart from the cyclical transmission of trypanosomosis by the *Glossina* spp. it is highly considered that mechanical transmission is a potential threat to livestock productivity in Ethiopia (Abebe and

Jobre, 1996). According to Sinshaw et al. (2006) prevalence of mechanically transmitted trypanosomosis was 6.1% in three districts bordering Lake Tana of Northwest Ethiopia.

Although fragmented reports are available on the prevalence of the disease, attempts have not been done to demonstrate the relative importance of tsetse and non-tsetse transmitted trypanosomosis. Virulence may vary not only because of the species and strain of the parasite but also due to the mode of transmission. For example, mechanical transmission of *Plasmodium chabaudi* (Spence et al., 2013) and *T. brucei* (Turner et al., 1995) in mice by needle passage increases virulence compared to transmission through the insect vector. In Northwest Ethiopia regardless of knowing the virulence of trypanosomes, trypanocidal drug treatment is administered frequently for the control of the disease (Dagnachew et al., 2005; Cherenet et al., 2006; Sinshaw et al., 2006). Data on the virulence of the parasite transmitted cyclically and the one purely transmitted mechanically is important in evaluating treatment responses and host resistance/tolerance to the infections as well as the distribution of the problem.

1.2.2. Control efforts against trypanosomosis in Ethiopia

Various efforts of control of the disease and losses have been directed mainly against the parasite through trypanocidal drugs and the vector through odour-baited and insecticide-impregnated targets/traps and insecticide-treated cattle (Slingenbergh, 1992; Leak et al., 1996). Recently vector control operations have been implemented mainly through specifically designed joint projects that offered some promising local results (Lemecha et al., 2006). However, the vector control operation is limited in areas of approximately 14% of the total tsetse-infested area. Moreover, recent situations of tsetse fly advances to previously unoccupied sites and development of trypanocidal drug resistance are thought to hamper the envisaged results of these efforts.

1.2.3. Trypanocidal drug resistance in Ethiopia

In Ethiopia, currently the most widely used trypanocidal drugs for *T. congolense* and *T. vivax* infection are ISM and DA. The occurrence of drug resistant particularly the non-tsetse transmitted trypanosome across Ethiopia is not well known. Trypanocidal resistance particularly against *T. congolense* infection is reported in the Ghibe Valley (Codjia et al., 1993; Mulugeta et al., 1997; Afework et al., 2000; Tewolde et al., 2004; Shimelis et al., 2008; Moti et al., 2012). In general, most of the studies conducted in Ethiopia to assess the therapeutic and prophylactic efficacy of trypanocidal drugs have involved *T. congolense*. Information is scanty especially comparative studies conducted to see whether there is a difference in resistance between *T. vivax* from tsetse infested and tsetse free areas.

1.3. Aims of the Study

Control of trypanosomosis and its cyclical vectors using various methods in tsetse infested regions of Ethiopia is being intensified. However, less attention has been given to the disease in

the non-tsetse infested areas, especially in the Northwestern part. This could be due to the fact that, in tsetse-infested areas, the combined effects of both cyclically and mechanically transmitted trypanosomes is more visible than in non-tsetse situation consequently underestimating the pathologic and economic impacts of the latter. On the other hand, fly control efforts and frequent use of trypanocides may significantly reduce the prevalence and intensity of the more prolific *T. congolense* leaving higher opportunity for *T. vivax* which can also be mechanically transmitted by other biting flies and which by its nature is difficult to detect in blood especially in chronic cases. Moreover, because of the significant attention given to tsetse transmitted trypanosomosis, it is expected that there is a high risk of trypanocidal resistance by *T. vivax* in this areas as a result of frequent and indiscriminate use of the available drugs. This assumption is further substantiated by the absence of any control effort, apart from treating clinical cases, against the non-tsetse transmitted trypanosomosis outside the tsetse belt. Therefore, it is hypothesized that *T. vivax* in tsetse infested areas, being under high pressure of trypanocidal treatment, develops more resistance to the commonly used trypanocides than those in non-tsetse infested areas. It is also assumed that although attention has not been given to control the mechanically transmitted trypanosomes in non-tsetse infested area, *T. vivax* in both tsetse and non-tsetse infested areas are equally important pathologically as they are also resulting in clinical cases and treatments often results clinical cures.

Therefore, the general objective of this PhD is to investigate and compare the pathogenicity and drug resistance of *T. vivax* isolates originated from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Specifically the following aims have been set.

1. To estimate the prevalence of bovine trypanosomosis and assessment of trypanocidal drug utilization practices in tsetse infested and non-tsetse infested areas of Northwest Ethiopia.
2. To investigate the pathogenicity of different isolates of *T. vivax* in experimentally infected young Zebu cattle.
3. To compare the pathogenicity of different isolates of *T. vivax* derived from tsetse-infested and non-tsetse infested areas in experimentally infected young Zebu cattle.
4. To investigate the presence of trypanocidal drug resistance of *T. vivax* isolates from tsetse and non-tsetse infested areas against DA and ISM at a lower and higher recommended doses in experimentally infected young Zebu cattle.

The major findings of this thesis are presented in different chapters, namely: Chapter II deals with prevalence of bovine trypanosomosis and assessment of trypanocidal drug utilization practices in tsetse infested and non-tsetse infested areas of Northwest Ethiopia, Chapter III discusses the experimental pathogenicity studies on *T. vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia, Chapter IV focusses on the experimental trypanocidal drug resistance studies of *T. vivax* isolates from tsetse and non-tsetse infested areas of Northwest Ethiopia at different doses of DA and ISM and Chapter V addresses general conclusions and perspectives.

Chapter II:

Prevalence of Bovine Trypanosomosis and Assessment of Trypanocidal Drug Utilization Practices in Tsetse Infested and Non-Tsetse Infested Areas of Northwest Ethiopia

Adapted from:

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Abstract

The Northwestern region of Ethiopia is affected by both tsetse and non-tsetse transmitted trypanosomosis. In the present work, questionnaire survey and cross-sectional study were undertaken to assess trypanocidal utilization practices and estimate the prevalence of bovine trypanosomosis in tsetse and non-tsetse infested areas of Northwest Ethiopia. Buffy coat technique and thin blood smear were employed for detecting trypanosomes, measurement of packed cell volume (PCV) and species identification. Blood from cattle 640 (324 and 316) and 795 (390 and 405) was examined in the months of October 2011 and 2012 from tsetse infested non-tsetse infested areas respectively. The questionnaire survey showed trypanosomosis was a significant animal health constraint for 84% (n=84) and 100% (n=100) of the farmers questioned in non-tsetse and tsetse infested areas of Northwest Ethiopia respectively. Responses on trypanocidal drug utilization practices indicated that risk factors for drug resistance are prevalent and treatment failures are common. Accordingly, majority of farmers in tsetse infested areas get trypanocides from drug stores and unauthorized sources whereas those from non-tsetse areas get from veterinary clinics. Moreover, treatment administration is mainly by animal health personnel and treatment frequency is a maximum of three times/year/animal for farmers in non-tsetse area whereas, it is administered mainly by the farmers more than seven times/year/animal in tsetse infested area. In tsetse infested areas, the prevalence of trypanosomosis varied from 17.59% in 2011 to 25.0% in 2012 and the difference was significant (P=0.023). Similarly, in non-tsetse infested area the prevalence varied from 3.85% in 2011 to 5.93% in 2012 but without significant rise. In both years, the prevalence was significantly higher ($P < 0.001$) in tsetse infested area (21.25%) than the non-tsetse infested area (4.91%). *Trypanosoma congolense* (75 %) was the most prevalent followed by *T. vivax* (20.58%) and mixed infections (4.41%) in tsetse infested area while in non-tsetse infested area only *T. vivax* was detected. The overall mean PCV in parasitaemic animals (20 ±2.3 SD) was significantly ($P < 0.001$) lower than that of aparasitaemic animals (27 ±4.3 SD). In conclusion, trypanosomosis is widely prevalent in both study areas and is causing significant anaemia. Farmers' trypanocidal utilization practices appear to pose risks of drug resistance. Therefore, the findings suggest further studies on the impact of the problem and efficacy of drugs circulating in both tsetse and non-tsetse infested areas of Northwest Ethiopia.

2.1. Introduction

In Ethiopia trypanosomosis is a serious constraint to livestock production and agricultural development. Due to the advancement of tsetse flies into formerly free areas, an estimated 220,000 km^2 areas is presently affected by tsetse flies (Abebe, 2005). The most prevalent trypanosome species are *T. congolense* and *T. vivax*. Various authors reported that the prevalence of trypanosomosis in tsetse infested areas range from 11.85-37% (Rowlands et al., 1993; Abebe and Jobre, 1996; Dagnachew, et al., 2005; Cherenet et al., 2006; Degu et al., 2012; Fikru et al., 2012). In non-tsetse infested areas of Northwest Ethiopia, the prevalence of trypanosomosis was in the range of 2-9% (Eneyew and Abebe, 1996; Cherenet et al., 2006; Sinshaw et al., 2006). Control of trypanosomosis in Ethiopia relies on trypanocidal drugs with

diminazene aceturate (DA) and isometamidium chloride (ISM) being drugs frequently employed. Consequently, drug resistance has become a common report particularly for *T. congolense* in tsetse infested areas (Afework et al., 2000; Tewolde et al., 2004; Shimelis et al., 2008; Moti et al., 2012). Although fragmented reports are available on the prevalence of the disease, attempts have not been done to demonstrate the relative importance of tsetse and non-tsetse transmitted trypanosomosis. Moreover, trypanocidal drug utilization practices have not been assessed to show risk factors for the development of drug resistance in the two areas. Therefore, this study was conducted with the aim of assessing risk factors for the development of trypanocidal drug resistance and estimate the prevalence of bovine trypanosomosis in tsetse and non-tsetse infested areas of Northwest Ethiopia.

2.2. Materials and Methods

2.2.1. Study areas

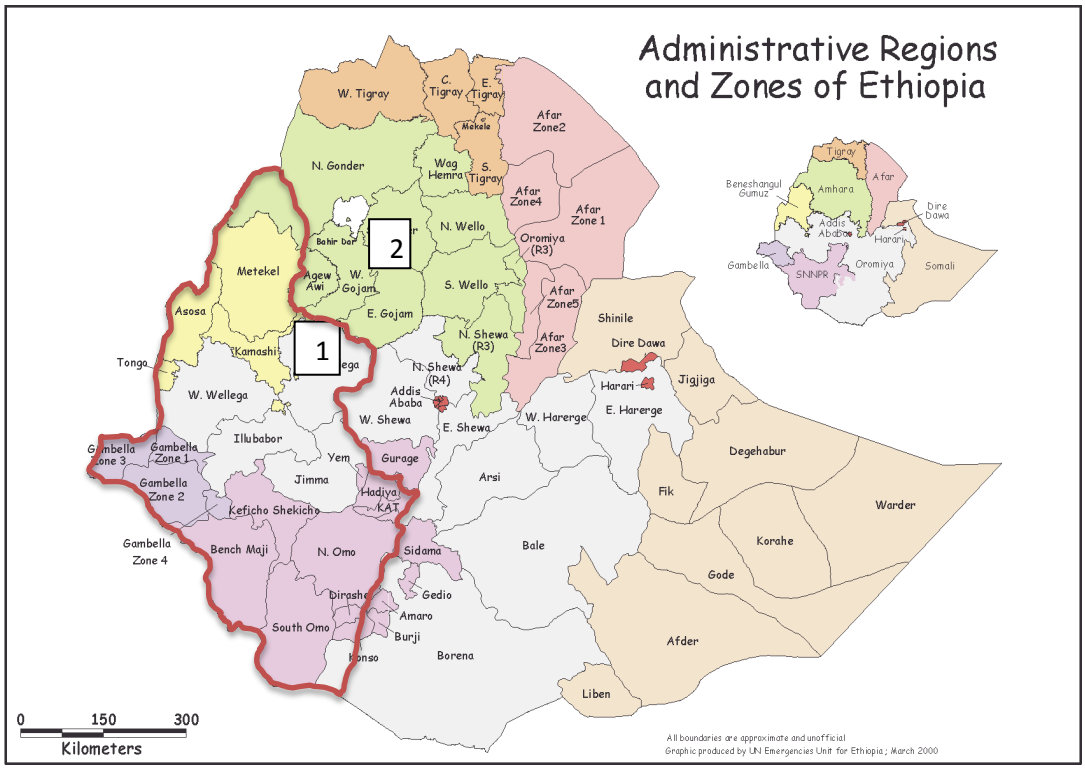


Figure 2.1. Map of administrative regions of Ethiopia and administrative zones of regions showing the study areas: tsetse infested area (Jabitehenan district of West Gojjam zone: 1) and non-tsetse infested area (Bahir Dar Zuria district of West Gojjam zone: 2). Tsetse infected area of Ethiopia is encircled in red. (Source: Modified from World Food Program Vulnerability Analysis and Mapping Unit, Ethiopia, July 1998).

The study was conducted in two districts, one from tsetse infested (Jabitehenan district) and one from non-tsetse infested (Bahir Dar Zuria district) areas of Northwest Ethiopia (Figure 2.1). Jabitehenan is located between 10° 30′ 42″ and 37° 7′ 11″ with altitude range from 1100-1500

meter above sea level (m.a.s.l.) whereas Bahir Dar Zuria is located 11° 36´ 0″ N and 37° 22´ 60″ E with altitude range from 1800-2000 m.a.s.l. The climate alternates with long summer rain fall (June - September) and a winter dry season (October - May) with mean annual rain fall of 1569.4 mm and the mean temperature varies between 16.7°C - 31.6°C. The areas are occupied by cultivated land, grazing land, forest shrub, bush and woodland, water bodies and the remaining is engaged by settlement population. The livestock population includes cattle, sheep, goat and equines which are an integral part of the livelihood of the people. Cattle are particularly important in the agricultural activities where the farmers are dependent on oxen power for crop production (CSA, 2010).

2.2.2. Study methodology

2.2.2.1. Questionnaire survey

A questionnaire survey was conducted to gather data on the problems of trypanosomes and practice of trypanocidal drugs usage in the study areas. To address the questionnaire survey a total of 200 farmers 100 in each district were interviewed with a structured questionnaire format. The interviewed peoples were selected randomly from the study areas during the prevalence study.

2.2.2.2. Cross-sectional study

Cross-sectional studies were conducted in October 2011 and October 2012 for the determination of the prevalence of bovine trypanosomosis as well as measurement of packed cell volume (PCV) values between parasitaemic and aparasitaemic animals in the study areas.

2.2.2.2.1. Study animals

The study animals included indigenous Zebu cattle populations found in the two study districts. The husbandry system depends on natural grazing and crop residues and kept in a traditional communal management system. Animals obtain water in the rainy season from seasonal rivers while in the dry season from perennial rivers flowing long in their locality.

2.2.2.2.2. Sampling method and sample size determination

A stratified multistage random sampling method was applied according to (Thrusfield, 2007). Two districts were selected from Amhara National Regional State (first stage) to represent tsetse infested and non-tsetse infested areas of Northwest Ethiopia (Figure 2.1). Then lists of Peasant Association's (PA's) within districts were compiled from a data obtained in the district's agricultural office (second stage). Herds selected within the same grazing land were considered as strata and representative numbers of animals were sampled. Parameters like sex, age, sampling period and areas were recorded for each individual animal during sample collection. Age was categorized into two (< 2 years and > 2 years) groups. The sample size was

determined based on the expected prevalence rate of 20% and 10% in tsetse infested and non-tsetse infested areas respectively, with an absolute desired precision of 5% at confidence level of 95%. Therefore, a total of 1435 cattle of which 640 (324 in 2011 and 316 in 2012) from tsetse infested area and 795 (390 in 2011 and 405 in 2012) from non-tsetse infested area) were examined in the months of October respectively.

2.2.2.2.3. Parasitological examination and PCV determination

Paired blood samples were collected from auricular vein of each animal using two haematocrit capillary tubes that were filled 3/4 of the height and sealed with Cristaseal. The capillary tubes were centrifuged at 12000 rpm for 5 minutes first to measure the PCV. Thereafter, the capillary was cut 1mm below the buffy coat to include the top layer of red blood cells, and the content of the capillary tube was expressed onto a clean slide, mixed and covered with a 22x22 mm coverslip (Paris et al., 1982). Then the slide was examined for trypanosomes based on their movement in the microscopic field. Confirmation of trypanosome species by morphological characteristics was done after staining with Giemsa by examination with oil immersion microscopy with 100x power of magnifications (Murray et al., 1977).

2.2.2.2.4. Isolation of T. vivax parasites

Isolation of *T. vivax* parasites were carried out during each sampling period. The isolates were collected from mono-infected animals with *T. vivax* using a parasitological technique (Paries et al., 1982) in the field. In the first survey, the isolates were collected in stabilates form from naturally infected cattle in the field in cryovials containing cryomedium and stored in liquid nitrogen (-196°C). In the second survey, the isolates were collected after purchasing naturally infected cattle from the field and transported into fly proof animal facility at the College of Veterinary Medicine and Agriculture of Addis Ababa University, and then stabilates were prepared from these animals at peak parasitaemia based on "rapid matching method" (Herbert and Lumsden, 1976). The cryomedium used for the preparation of stabilates was 8% glycerol. The stabilates were prepared by mixing equal volume (1:1) of infected blood with cryomedium. These stabilates were passaged into goats and calves to see the typical motility and morphological structure of *T. vivax* using the parasitological technique (Paries et al., 1982), and tested with PCR (Masiga et al., 1992) to confirm pure *T. vivax.*

2.2.3. Data analysis

Data was recorded during sample collection, parasitological examination and PCV measurement as well as questionnaire responses into Excel Spread Sheets to create a database and imported to SPSS version 20 for analysis. Descriptive statistics, Student's *t*-test and Logistic regression were used to explain results and compare variables. Logistic regression on the prevalence of trypanosomes was performed on the variables; sampling years, sampling areas, sex and age groups. Student's *t*-test was employed to compare the mean PCV of the

parasitaemic animals with that of the aparasitaemic animals. Significant level was set at $P < 0.05$.

2.3. Results

2.3.1. Questionnaire survey

All the respondents in tsetse infested and non-tsetse infested areas rear cattle primarily for draught purpose and income generation. Communal or free grazing is the major livestock management practice in the study areas. Trypanosomosis was ranked as the first animal health constraint and known for the last two decades by 100% and 84% of the respondents from tsetse infested and non-tsetse infested areas, respectively. Control of trypanosomosis in both areas relies mainly on treatment of animals with trypanocidal drugs; isometamidium chloride (ISM) and diminazene aceturate (DA). The vast majority of respondents (90%) from non-tsetse infested areas get trypanocidal drugs from veterinary clinics and drug shops whereas most respondents (82%) in tsetse infested areas ascertained that their drug sources were drug stores and unauthorized shops (Figure 2.2A). On the other hand, most farmers in non-tsetse infested area send their cattle to veterinary clinics or animal health posts for trypanosomosis cases whereas majority of those in tsetse-infested area administer trypanocidal drugs by themselves or family members (Figure 2.2B). According to the questionnaire survey respondents, 40%, 20% and 40% of them use DA, ISM and both respectively in non-tsetse infested areas as compared to 2%, 30% and 68% in tsetse infested areas. However, the drug of their choice was DA in non-tsetse infested areas (52%) and ISM in the tsetse infested areas (94%). Eighty percent of respondents in tsetse infested areas treat their animals more than seven times/per year/animal whereas 80% of those in non-tsetse infested area give a maximum of three injections per year/animal (Figure 2.2C). Trypanocidal treatment failures were reported by 66% and 94% of the respondents in non-tsetse and tsetse infested areas respectively.

2.3.2. Parasitological findings

Out of the total 640 and 795 animals examined using parasitological techniques respectively from tsetse-infested and non-tsetse-infested areas, 21.25% and 4.91% ($P < 0.001$, 95%CI: 2.367-11.517) were found positive for the disease. In tsetse infested areas, the prevalence was significantly higher in 2012 compared to the finding in 2011 whereas no significant variation was observed between the two sampling years in non-tsetse infested area (Table 2.1). Among the trypanosome species identified in both study period, significantly higher prevalence was detected for *T. congolense* compared to *T. vivax* and mixed infections. Generally, the prevalence was significantly higher in male than female animals and in adults than young animals in tsetse infested areas while such difference was not significant in the non-tsetse infested areas. When *T. vivax* was considered alone, the prevalence between tsetse infested (4.38%) and non-tsetse infested (4.91%) areas was not significantly different.

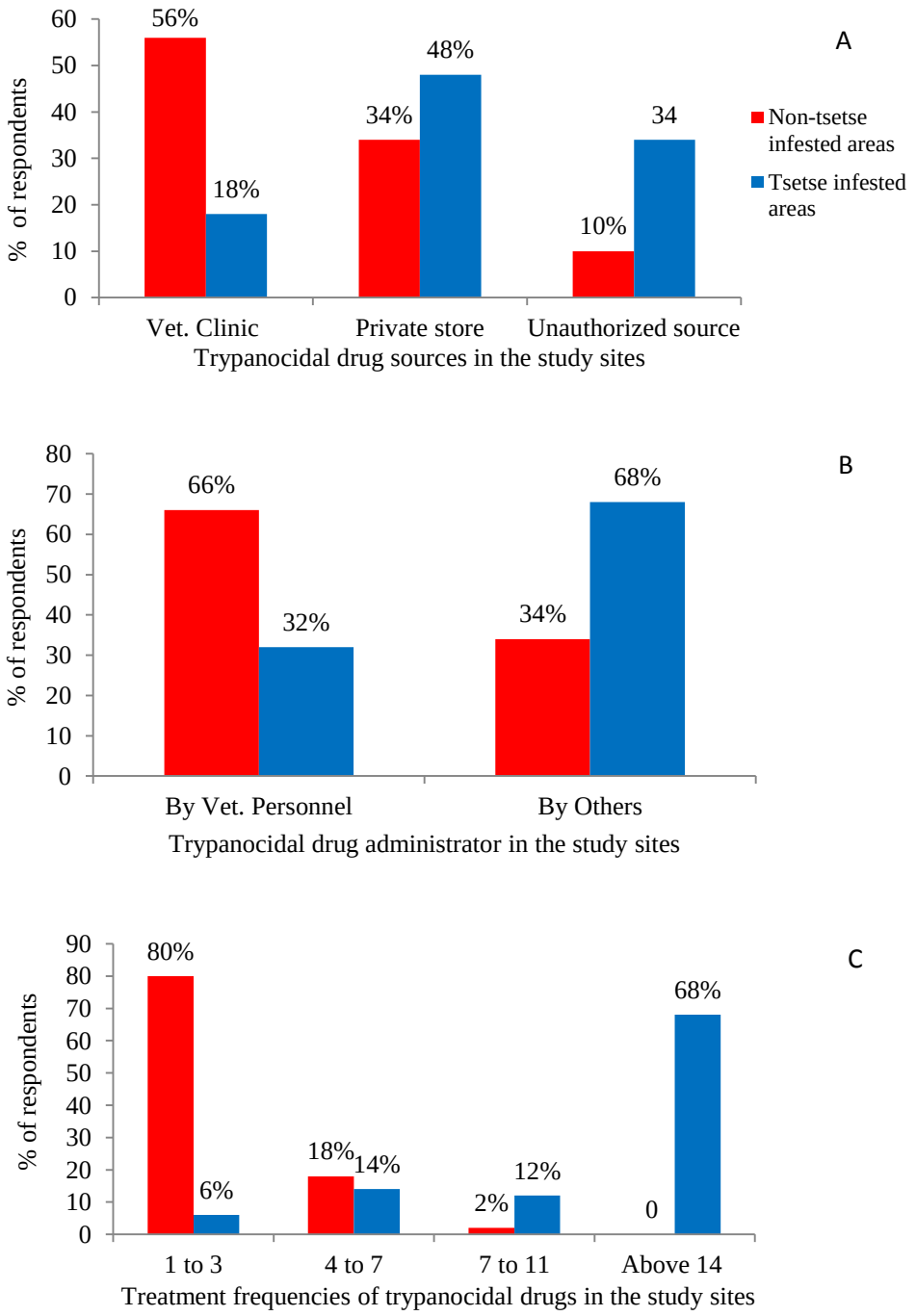


Figure 2.2. Questionnaire response on trypanocidal drug utilization practices in the study areas. (A) Sources of trypanocidal drugs for treatment, (B) administration of trypanocidal drugs in cattle, (C) trypanocidal drug treatment frequencies/animal/year.

Table 2.1. Prevalence of bovine trypanosomosis in tsetse infested and non-tsetse infested areas of Northwest Ethiopia in 2011 and 2012.

Parameters	**Group**	**% Positive in 2011**	**% Positive in 2012**	**95%CI b/n years**	
Parasite detection	Positive (tve) TT	17.59 (N=324)[a]	25.0 (N=316)[b]	0.437	0.939
	Positive (tve) NT	3.85 (N=390)[c]	5.93 (N=405)[c]	0.328	1.228
Parasite species	Tc TT	13.27 (N=324)[a]	18.67 (N=316)[b]	0.421	0.995
	Tv TT	3.70 (N=324)[c]	5.06 (N=316)[c]	0.309	1.436
	M TT	0.62 (N=324)[c]	1.27 (N=316)[c]	0.081	2.445
	Tv NT	3.85 (N=390)[c]	5.93 (N=405)[c]	0.328	1.228
Sex	Male tve TT	10.80 (N=173)[a]	19.30 (N=193)[b]	1.339	3.042
	Female tve TT	6.79 (N=151)[a]	15.69 (N=123)[b]		
	Male tve NT	2.11 (N=194)[c]	2.96 (N=196)[c]	0.577	2.091
	Female tve NT	1.79 (N=196)[c]	2.96 (N=209)[c]		
Age	Young tve TT	1.85 (N=80)[a]	7.28 (N=113)[b]	1.077	2.674
	Adult tve TT	15.74 (N=244)[c]	17.72 (N=203)[c]		
	Young tve NT	1.28 (N=133)[a]	0.74 (N=93)[a]	0.710	3.470
	Adult tve NT	2.56 (N=257)[a]	5.19 (N=312)[a]		

Tc-*Trypanosoma congolense*, Tv-*Trypanosoma vivax*, M-mixed (Tc and Tv), TT-tsetse infested area, NT-non-tsetse infested area, tve-positive. In each parameter of variables, superscripts with different letters indicate significant differences between values at $P < 0.05$.

2.3.3. Isolation of *T. vivax* parasites

Isolation of *T. vivax* parasites were carried out during each sampling periods. In the first survey (October 2011) a total of 14 *T. vivax* isolates, 10 from tsetse infested areas and 4 from non-tsetse infested areas while in the second survey (October 2012) a total of 7 *T. vivax* isolates, 4 from tsetse infested areas and 3 from non-tsetse infested areas were collected. The stabilates from the second survey were passaged into calves and goats, and develop infections and showed the typical motility and morphological structure of *T. vivax* using parasitological technique (Paries et al., 1982), and confirmed as pure *T. vivax* with PCR (Masiga et al., 1992). These stabilates were named as TT-ETBS1, TT-ETBS2, TT-ETBS3 and TT-ETBS4 for tsetse infested area and NT-ETBD1, NT-ETBD2 and NT-ETBD3 for the non-tsetse infested area. From these isolates the first two isolates (TT-ETBS1 and TT-ETBS2) in tsetse infested area and all the three isolates (NT-ETBD1, NT-ETBD2 and NT-ETBD3) in the non-tsetse infested area were used for experimental studies. The abbreviation indicated in the name of isolates stand as follows (TT - tsetse infested area, NT - non-tsetse infested area, ET - Ethiopia, BS – Birsheleko area of Jabitehenan distirict, BD-Bahir Dar Zuria district) i.e. *T. vivax* isolated from Ethiopia at Birsheleko areas of Jabitehenan district for tsetse infested area and at Bahir Dar Zuria district for non-tsetse infested area and the numbers are the sequential order of the isolates. Stabilates collected in the first survey were failed to develop infection in passaged animals. The possible reason for failure to develop infection could be the level of parasitaemia was low during the preparation of stabilates in the field.

2.3.4. Haematological findings

The haematological findings of the examined animals were investigated by comparing the PCV

values with respect to trypanosome infection, species of trypanosomes, sampling sites and years. Consequently, overall mean PCV was significantly reduced in parasitaemic animals compared to aparasitaemic animals ($P < 0.001$) when samples were pooled from both study sites and study years. Similarly, irrespective of trypanosome status, mean PCV was significantly lower ($P < 0.001$) in tsetse infested area compared to non-tsetse infested area. However, the mean PCV values were not significantly different between sampling years (Table 2.2). In tsetse infested areas, the mean PCV was significantly lower ($P < 0.001$) in parasitaemic animals compared to that of aparasitaemic animals (Table 2.3) in both study years. A similar trend was noticed in non-tsetse infested areas. The decrease in mean PCV was similar when data were sorted according to parasite species that the animal was harboring which was also true for those in non-tsetse areas compared to values for apparently aparasitaemic animals.

Table 2.2. Mean PCV of animals examined for trypanosomosis in both tsetse and non-tsetse infested areas of Northwest Ethiopia irrespective of trypanosomosis status.

Parameters	**Group**	**Number examined**	**Mean PCV**	**SD**	***P*-value**
Parasite detection	Positive	175	20	4.1	0.00
	Negative	1260	27	4.3	
Sampling years	2011	714	26	4.7	0.05
	2012	721	26	4.9	
Sampling areas	Tsetse area	640	25	5.4	0.00
	Non-tsetse area	795	27	4.2	

Table 2.3. Mean PCV of parasitaemic and aparasitaemic animals in tsetse infested and non-tsetse areas of Northwest Ethiopia during the study period.

Parameters	**Group**	**No examined**	**Mean PCV**	**SD**	***P*-value**
Parasite detection in 2011	Positive TT	57	20^{a}	3.7	0.00
	Negative TT	267	27^{b}	4.9	
	Positive NT	15	21^{a}	3.3	0.00
	Negative NT	375	27^{b}	3.8	
Parasite detection in 2012	Positive TT	79	20^{a}	4.7	0.00
	Negative TT	237	26^{b}	4.7	
	Positive NT	24	19^{a}	3.6	0.00
	Negative NT	381	27^{b}	3.9	
T. vivax detection in both years	Positive TT	28	21^{a}	4.1	0.09
	Positive NT	39	20^{a}	3.5	

TT-tsetse infested area, NT-non-tsetse infested area. In each parameter of variables, superscripts with different letters indicate significant differences between values at $P < 0.05$.

2.4. Discussion

Bovine trypanosomosis and its impact is well known by the farmers in the study areas although the frequency of respondents was lower in non-tsetse area than in tsetse area. Similar observations have already been reported by Afework et al. (2000) from Metekel district of Northwest Ethiopia, Tewolde et al. (2004) from western Ethiopia and Dagnachew et al. (2005) from Northwest Ethiopia where the cyclical vector tsetse fly are present. Similarly Zewdu et al. (2013) at Baro-Akobo and Gojeb river basins has reported that 94.1% of the respondents considered bovine trypanosomosis as an economically important cattle disease which accounted for 64.6% of the total annual deaths in the year 2011/2012. To combat the problem, farmers reported to use diminazene aceturate (DA) and isometamidium chloride (ISM), the most commonly available trypanocidal drugs in the areas which is also similar for other places as reported by Zewdu et al. (2013). The majority of the respondents from non-tsetse infested areas preferred to use DA whereas those in tsetse infested areas preferred ISM. A similar preference in non-tsetse infested areas has already been reported by Van den Bossche et al. (2000) in the eastern province of Zambia. This could be linked to the fast treatment responses as in case of DA or longevity of protection from subsequent infections as in the case of ISM when given at a prophylactic dose.

Although the problem is significant in both study sites, trypanocidal treatment frequencies are higher and injections were mainly given by farmers or untrained personnel in tsetse infested areas as compared to those in non-tsetse areas. This finding is in agreement with various reports done in Ethiopia (Afework et al., 2000; Tewolde et al., 2004; Shimelis et al., 2008; Zewdu et al., 2013). Uilenberg (1998) reported that the number of treatments over a year reflects the magnitude of trypanosome challenge in an area. Moreover, most farmers in the tsetse area and significant number of them in non-tsetse area obtain trypanocidal drugs from drug stores and unauthorized sources with or without prescription. Altogether, farmers' practices suggest widespread prevalence of risk factors for emergence of trypanocidal drug resistance. Reasons for the misuse of trypanocidal drugs could be related to the inadequacies of veterinary services, the widespread availability of trypanocidal drugs in informal markets and frequent treatment failures that would force them to have the drugs at their back yard.

Trypanosomosis is a major constraint to the utilization of large land resources and also affect livestock, particularly cattle which have a major role in the agricultural economy of Ethiopia (Jemal and Hugh-Johns, 1995). In the present study the prevalence of bovine trypanosomosis was 21.25% in tsetse infested areas and 4.91% was in non-tsetse infested areas. This is in concordance with previous reports (Afework et al., 2000; Tewelde et al., 2004; Dagnachew et al., 2005; Cherenet et al., 2006; Shimelis et al., 2011). The higher prevalence of trypanosomosis in tsetse infested area compared to the non-tsetse area could be attributed to the dominance of *T. congolense* over *T. vivax*. Similar findings were reported in tsetse infested areas of Ethiopia, the dominant trypanosome species was *T. congolense* (Rowlands et al., 1995; Leak, 1999; Dagnachew et al., 2005). The predominance of *T. congolense* infection in cattle may be due to the high number of serodemes of *T. congolense* as compared to *T. vivax* and the development of

a stonger immune response to *T. vivax* (Gardiner, 1989). However, when animals infected with *T. vivax* alone were considered, prevalence was low and similar between tsetse infested and non-tsetse infested areas. This could be explained by the fast clearance of the infection by the host immune response rendering the East African strain less pathogenic as it was previously suggested (Hoar, 1972; Molyneux et al., 1983; Gardiner, 1989). It could also be possible that *T. vivax* in these areas establish a more chronic infection where conventional parasitological techniques fail to detect the presence of the parasite in the blood. The lower prevalence reported for *T. vivax* is in agreement with various workers who reported a prevalence range of 2% to 9% (Eneyew and Abebe, 1999; Abebe and Jobre, 1996; Cherenet et al., 2006; Sinshaw et al., 2006). This study has also revealed variations in the prevalence of the disease between two study years in the tsetse infested area. Although, fly survey was not carried out in the present study, the variation might be attributed to changes in vector fly densities or infection rates.

Trypanosome infections were more prevalent in males and adult cattle than in females and young ones. This agrees with the following findings (Trail et al., 1994; Magona et al., 2008; Daya and Abebe, 2008; Dayo et al., 2010). The higher prevalence in male animals might be associated to the frequent exposure of male animals to the bites of vectors during traction period. In contrast to our findings Rowlands et al. (1995) and Okech et al. (1996) reported that the infection was more important in females than in males. The age difference could be linked to reproduction and work stresses to which most adult cattle are exposed and the frequency of exposure for vectors of tsetse flies.

The fact that animal trypanosomosis causes significant anaemia (Murray and Dexter, 1988; Cherenet et al. 2006; Dagnachew et al., 2005, Sinshaw et al., 2006; Degu et al., 2012) was confirmed in this study. Measuring the mean PCV value is one of the indicators of a herd infected with trypanosomosis and hence the anaemic status of infected animals in the present findings showed reduction of PCV values in both study areas. Rowlands et al. (2001) in Ghibe observed that with a decrease in the PCV value, the proportion of infected animals increased and hence the mean PCV was a good indicator for the health status of herds in trypanosomosis endemic areas. The fall in mean PCV was regardless of the trypanosome species detected in those parasitological positive animals. Moreover, very comparable lower PCV values were noticed in those animals infected with *T. vivax* from both study sites; altogether suggesting the pathologic significance of *T. vivax* in both tsetse and non-tsetse infested areas of Northwest Ethiopia.

In conclusion, the questionnaire survey underlined trypanosomosis is a major animal health constraint in both tsetse and non-tsetse infested areas of Northwest Ethiopia and trypanocidal drugs available in the area are exposed to serious risks of drug resistance. The cross-sectional studies conformed that bovine trypanosomosis is causing significant reduction in the mean PCV of parasitaemic animals in both areas. *T. congolense* and *T. vivax* were species of trypanosomes responsible for the occurrence of trypanosomosis in tsetse infested areas whereas only *T. vivax* species was identified in non-tsetse infested areas. However, the prevalence of *T. vivax* infection and associated fall in PCV was similar between the two study areas suggesting the presence of

similarities in pathological consequences. Therefore, employing an integrated control program against the parasite and the vector is required in both areas and further studies on trypanocidal drug efficacy and pathogenicity of *T. vivax* is essential.

Chapter III:

Experimental Pathogenicity Studies on *Trypanosoma vivax* Isolates from Tsetse Infested and Non-Tsetse Infested Areas of Northwest Ethiopia

This chapter is divided into three components of the experimental works**:**

3.1. Comparative clinical, haematological and pathological observations in young Zebu (*Bos indicus*) cattle experimentally infected with *T. vivax* from tsetse infested and non-tsetse areas of Northwest Ethiopia.

3.2. Comparative biochemical changes in young Zebu (*Bos indicus*) cattle experimentally infected with *T. vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia.

3.3. Immune cytokines responses in young Zebu (*Bos indicus*) cattle experimentally infected with *T. vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia.

3.1. Comparative clinical, haematological and pathological observations in young Zebu (*Bos indicus*) cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia

Adapted from:

Dagnachew, S., Bezie, M., Terefe, G., Abebe, G., Barry, D.J., Goddeeris, B.M., 2015. Comparative clinical and haematological observations in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse areas of Northwest Ethiopia. Acta Veterinaria Scandinavica **57**, 24.

Abstract

The objective of this study was to determine and compare clinical, haematological and pathological findings in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. In the first experiment eighteen cattle purchased from a trypanosome free area (Debre Brehan: Northcentral Ethiopia) aged between 9 and 12 months were assigned randomly into three groups of six animals (group TT-ETBS1= infected with a *T. vivax* isolate 1 from tsetse infested area, group NT-ETBD1 = infected with a *T. vivax* isolate 1 from non-tsetse infested area and group NIC = non-infected control). Similarly, in the second experiment sixteen animals divided into four groups of four animals (group TT-ETBS2= infected with a *T. vivax* isolate 2 from tsetse infested area, group NT-ETBD2= infected with a *T. vivax* isolate 2 from non-tsetse infested area and group NT-ETBD3 = infected with a *T. vivax* isolate 3 from non-tsetse infested area and NIC-non-infected control). All experimental animals were kept in a fly proof animal unit and acclimatized for 1 month prior to the beginning to the experiment. For each experimental animal 2 mL of blood taken from infected donor calves respective of the group was inoculated intravenously ~ at 10^6 trypanosomes/mL except the NIC groups while in the second experiment the control group received 2 mL of non-infected blood from a donor calf. During the study period clinical examination was performed daily while parasitaemia and haematological analysis: packed cell volume (PCV), haemoglobin concentration (Hgb), total red blood cell (RBC) and white blood cell (WBC) counts and blood indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH] and mean corpuscular hemoglobin concentration [MCHC]) were determined in a week interval until the end of the experiment. Postmortem examinations were done in euthanized animals during the infection period on those animals showing severe clinical manifestations, and on all animals at the end of the experiment. The infection was clinically characterized by reduced feed intake, weakness, pyrexia, parasitaemia, rough hair coat, enlarged prescapular lymph nodes, lacrimation, weight loss, pallor mucus membrane and dehydration. Less frequently diarrhea, oedema, nervous signs and recumbence were observed in all groups of the infected animals. In the second experiment, body weight in infected groups was significantly lower than that of the non-infected control. Similarly body weight loss was higher ($P < 0.001$) in animals infected with tsetse infested isolate than the non-tsetse infested isolates. Parasitaemia appearing in circulation on 6 and 12 days post-infection (dpi) for NT and TT infected cattle and on 4 and 7 dpi for NT and TT infected cattle in the first and second experiment respectively. The mean PCV values were significantly lower ($P < 0.001$) in all infected groups than non-infected groups in both experiment. In the second experiment in addition to the mean PCV other haematological values including Hgb, total RBC and WBC counts were measured and found to be lower ($P < 0.001$), and mean MCV was higher ($P = 0.01$) in all infected groups than non-infected control at different time points during the study period. Moreover, the decrease in haematological values was higher ($P < 0.05$) in NT groups than the TT group. At necropsy, infected animals showed enlarged and haemorrhagic spleen; swollen, oedematous and enlarged lymph nodes; pneumonic and emphysematous lung; enlarged liver with rounded edge, and haemorrhagic lesions on the brain and intestine. The postmortem examination of the animals in the control group did not

reveal any significant gross lesion. The histopathological examination revealed significant abnormalities on major organs, characterized by lymphoid hyperplasia in the white and red pulp of the spleen, interstitial pneumonia, tubulointerstitial nephritis, multifocal myocarditis, meningoencephalitis, lymphoid hyperplasia in lymph nodes, and mononuclear cell infiltration, necrosis and haemosiderosis of the hepatic tissue. In conclusion, *T. vivax* parasites originating from both areas showed significant virulence factors leading to the development of clinical trypanosomosis, reduction in haematological values as well as gross and histopathological lesions. However, the appearance of parasitaemia was earlier in the NT compared to TT infected groups in both experiments. In addition higher body weight loss in TT than NT infected groups and higher reduction in haematological values in the NT infected groups than TT group were noticed in the second experiment. Therefore, the pathogenicity of *T. vivax* from the non-tsetse infested area can be considered as nearly as important as that of its counterpart derived from the tsetse infested area.

3.1.1. Introduction

The pre-patent period of bovine trypanosomosis is usually 1 to 3 weeks, depending on the virulence of the infecting trypanosome, the infective dose and the immune status of the host. Experimental infection with African trypanosomes typically follows three successive stages: acute, stabilization and chronic. The early acute phase of the disease is characterized by the continuous presence of trypanosomes in the blood at detectable concentrations. Fever is highest at the first peak of parasitaemia and fluctuates thereafter with parasitaemia waves. With the onset of parasitaemia, anaemia develops. Other signs associated with trypanosomosis include pallor of the mucous membranes, enlargement of lymph node and spleen, weakness, lethargy, loss of condition, abortion and reduced milk production (Taylor and Authié, 2004).

The main haematological changes observed in natural cases of bovine trypanosomosis due to *T. vivax* infections are reported to be anaemia associated with decrease in PCV, haemoglobin and RBCs counts, and severe leucopenia (Silva et al., 1999). Similarly, blood cellular damage and histopathological changes in liver, kidney, spleen and other related organs were found in cattle experimentally infected with trypanosomes (Kadima et al., 2000). Information related to the impact of *T. vivax* infections on haematological values as well as clinico-pathological abnormalities is scarce in Ethiopia. Moreover, the relative importance of the tsetse adapted and the mechanically transmitted *T. vivax* in terms of pathological parameters has not been investigated. For better understanding of the pathogenicity of *T. vivax* from tsetse and non-tsetse infested areas of in Northwest Ethiopia, the present study was therefore carried out to determine and compare clinical findings, haematological values and pathological changes in experimentally infected young Zebu cattle.

3.1.2. Materials and methods

3.1.2.1. Experimental setup

3.1.2.1.1. Experimental study site

The experimental studies were carried out in a fly-proof animal facility located in the premises of the College of Veterinary Medicine and Agriculture of Addis Ababa University at Debre Zeit, Ethiopia.

3.1.2.1.2. Experimental animals

A total of 34 animals, 18 for the first experiment and 16 for the second experiment of indigenous Zebu (*Bos indicus*) cattle, aged 9 to 12 months, were purchased from a trypanosome free area (Debre Brehan: Northcentral Ethiopia) located at 9°4´N and 39°32´E with an altitude of 2840 m.a.s.l. about 130 km north of Addis Ababa. The animals were transferred into a fly-proof experimental animal house of the College of Veterinary Medicine and Agriculture of Addis Ababa University at Debre Zeit located at 9°6'N and 37°15'E with an altitude of 1920 m.a.s.l. about 47 km east of Addis Ababa. Animals were ear-tagged, examined for the presence of trypanosomes and other blood parasites using blood smear technique (Paries et al., 1982) and faecal egg count method (Soulsby, 1982) for helminthes. To avoid occurrence of pneumonia associated with transport stress and change of environment, all animals were treated on arrival with oxytetracycline 20% w/v (Chongqing Fangtong Animal Pharmaceutical Co., Ltd, China). All animals were treated with albendazole 2500 mg bolus and Ivermectin (Chengdu Qiankum Veterinary Pharmaceuticals Co.Ltd., China) to control internal and external parasites. After treatment prior to the beginning of the experiment animals were acclimatized for one month for the new environment, handling and feeding conditions.

3.1.2.1.3. Feeding and animal management

Animals were fed grass hay and supplemented with concentrates of wheat bran and green Elephant grass. Water and mineral lick were freely available. The handling of animals during the experiment was based on international guiding principles for biomedical research involving animals proposed by the Council for International Organizations of Medical Sciences (1985/2012). The research was authorized by the Animal Research Ethics Review Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University (Permit No: VM/ERC/003/04/013). Animals suffering from severe infection with PCV below 15% and detectable nervous signs and recumbence during the study periods and all animals at the end of each experiment were euthanized by using overdose phenobarbitol sodium intravenous administration.

3.1.2.1.4. Experimental groups

In the first experiment, eighteen animals were assigned randomly into three groups of six animals: group TT- ETBS1 = infected with a *T. vivax* isolate 1 from tsetse infested area (Ethiopia Birsheleko isolate 1), group NT- ETBD1 = infected with a *T. vivax* isolate 1 from non-tsetse infested area (Ethiopia Bahir Dar isolate 1) and group NIC= non-infected control. In the second experiment, sixteen animals were divided in to four groups of four animals: group TT- ETBS2 = infected with a *T. vivax* isolate 2 from tsetse infested area (Ethiopia Birsheleko isolate 2), group NT- ETBD2 = infected with a *T. vivax* isolate 2 from non-tsetse infested area (Ethiopia Bahir Dar isolate 2), group NT-ETBD3 = infected with a *T. vivax* isolate 3 from non-tsetse infested area (Ethiopia Bahir Dar isolate 3) and group NIC= non-infected control received non-infected blood from a donor calf.

3.1.2.1.5. Origin of the parasites and animal challenge

The *T. vivax* isolates used for both experiments were originally collected from naturally mono-infected cattle in Jabitehenan district of Birsheleko area (TT-ETBS1 and TT-ETBS2) and Bahir Dar Zuria district (NT-ETBD1, NT-ETBD2 and NT-ETBD3) of tsetse and non-tsetse infested areas respectively of Northwest Ethiopia. Stabilates were prepared from these animals using 8% glycerol (1:1) and cryopreserved in liquid nitrogen (-196°C). The isolates were confirmed as pure *T. vivax* by a screening PCR (Masiga et al., 1992) and compared by targeting microsatellite markers using six different primers sets (Duffy et al., 2009), and found to be similar in four different primer sets. *T. vivax* stabilates were checked for their viability and then inoculated into donor calves for propagation to use for experimental infections. The establishment of infection and parasite burden per milliliter of blood was quantified using rapid matching method (Herbert and Lumsden, 1976) on donor animals. Therefore, all infected experimental groups received intravenously 2 mL of infected blood approximately at 10^6 trypanosomes/mL from donor calves respective of the group. The control group in the second experiment received 2 mL of blood from a non-infected donor calf.

3.1.2.2. Clinical and parasitological examinations

During the study period (November 2012 - January 2013 in the first experiment and March - May 2014 in the second experiment) all animals were examined daily for clinical parameters (appetite, rectal temperature, body condition, color of mucous membranes, size of peripheral lymph nodes, coat condition, faecal consistency, lacrimation, subcutaneous oedema etc.) at their pen. Visible mucus membranes, palpable lymph nodes, skin elasticity and other conditions were thoroughly inspected. Rectal temperature was taken in the morning time with a digital thermometer. Blood samples were collected using anti-coagulant coated vacutainer tubes, and examined for the presence of trypanosomes daily until the detection of parasites, and a week interval after parasitaemia was established using wet blood smears and buffy coat technique (Murray et al., 1977; Paris et al., 1982).

3.1.2.3. Haematological analysis

About 5 mL of blood was collected in a week interval during the study period from jugular vein of all the experimental animals using ethylene diamine tetra acetic acid (EDTA) coated vacutainer tubes. Haematological parameters measured include: PCV, haemoglobin (Hgb) concentration, total RBC and WBC counts, calculation of erythrocyte indices; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) (Coles, 1986). PCV was measured by haematocrit centrifugation technique using a Hawksley microhaematocrit reader. Total RBC and WBC counts were carried out manually using the improved Haemocytometer. Haemoglobin (Hgb) concentration was measured by the Sahili's Acid-Haematin method. Erythrocyte indices were also calculated from the above haematological values using the following formulas.

MCV = PCV x 10 / RBC

MCHC = Hgb conc. x 100 / PCV

MCH = Hgb conc. x 10 / RBC

3.1.2.4. Gross and histopathological examinations

Animals showed severe clinical signs (nervous signs, recumbence and PCV below 15%) and all animals at the end of the experiment were humanely euthanized using an overdose phenobarbitol sodium intravenous administration. Consequently, gross pathological abnormalities such as change in size, weight, color, consistency and texture were noted on each examined organs. Representative tissue samples were taken from lymph nodes, spleen, liver, heart, kidney, lung and brain, and fixed in 10% neutral buffered formalin for histopathological studies. Formalin fixed tissues were dehydrated in ascending grades of alcohol, cleared by two changes of xylene, paraffin embedded, sectioned at 3-5 μm thickness, and stained with haematoxylin and eosin for microscopic examinations (Bush, 1975). At high magnification, cellular changes such as macrophage and leucocyte infiltrations, cell and tissue damages were recorded.

3.1.2.5. Data analysis

The entire data was entered to Excel spreadsheet and imported to SPSS version 20 for statistical evaluation. Descriptive statistics was used to explain the clinico-pathological findings. Differences in mean haematological values measured between groups were analyzed by the General Linear Model repeated measure ANOVA. Significant level was set at $P < 0.05$.

3.1.3. Results

3.1.3.1. Parasitaemia and clinical findings

In the first experiment, parasitaemia was detected on 6 and 12 days post infection (dpi) in animals infected with *T. vivax* isolated from non-tsetse and tsetse infested areas respectively. Animals in group NT-ETBD1 showed early peak parasitaemia on day 8 compared to group TT-ETBS1 which showed on day 14. Infected animals remained trypanosome positive with fluctuating parasitaemia throughout the experimental period (Figure 3.1.1A). In the second experiment parasitaemia was detected on 4 and 7 dpi in animals infected with *T. vivax* isolated from non-tsetse and tsetse infested areas respectively. Animals in group NT-ETBD3 showed early peak parasitaemia on day 7 compared to groups TT-ETBS2 and NT-ETBD2 which showed on 14 dpi. Similarly, animals remained trypanosome positive with fluctuating parasitaemia throughout the experimental period. High fluctuations were seen in animals infected with non-tsetse isolates, while parasitaemia dropped uniformly after its peak for the tsetse infested isolate infected group (Figure 3.1.1B). Even though group NT-ETBD3 showed a sharp decline in parasitaemia after its peak on 7 dpi, it persisted being the highest from 35 to 56 dpi.

In the first experiment, all the infected cattle developed clinical trypanosomosis characterized by reduced feed intake, fever, rough hair coat, enlarged superficial lymph nodes (Figure 3.1.2A), congested mucus membrane, lacrimation and loss of body weight were early clinical findings whereas pallor of mucus membranes, dehydration and emaciation (Figure 3.1.2B) were predominant in the later stage of the infection. Less frequent clinical signs seen include dullness, diarrhea (Figure 3.1.2C), oedema (Figure 3.1.2D), corneal opacity, weakness, nervous signs and recumbence. The mean rectal temperatures of infected groups (39.07 ±0.64, 39.17 ± 0.70 for TT-ETBS1 and NT-ETBD1, respectively) were significantly higher ($P < 0.001$) than that of the non-infected control (38.39 ±0.30). The onset of rise in rectal temperature coincided with the appearance of parasitaemia. The rise in temperature was followed by fluctuations (Figure 3.1.2E). The highest mean temperature recorded during the study period was 40.5°C in NT-ETBD1 group on 10 dpi. Four infected animals (two from TT-ETBS1 and another two in NT-ETBD2 groups) showing severe clinical manifestations (PCV below 15%, nervous signs and recumbence) were euthanized using an overdose phenobarbitol sodium intravenous administration on 30 dpi.

Experiment two also demonstrated clinical trypanosmosis comparable to experiment one. The major clinical signs observed (reduced feed intake, enlarged lymph nodes, rough hair coat and pallor of mucus membranes) observed at each time points were added together and scored respective of the groups as indicated in Figure 3.1.3A. The mean rectal temperatures in °C of infected groups (39.07 ±0.64, 39.17 ±0.70, 39.22 ±0.70 for TT-ETBS2, NT-ETBD2, NT-ETBD3, respectively) were significantly higher ($P < 0.001$) than control group (38.39 ±0.30). The temperature of infected animals started rising from the fourth dpi coincided with the appearance of parasitaemia. The rise in temperature was followed by fluctuations (Figure

3.1.3B) during the study period. The highest mean temperature recorded during the study period was 40.15°C in NT-ETBD2 group on 7 dpi. Infected groups significantly lost body weight but the control group kept on gaining weight ($P < 0.001$). Body weight loss was also significantly higher ($P < 0.001$) in TT-ETBS2 infected group as compared to NT-ETBD2 and NT-ETBD3 infected groups. This decrease in body weight in infected animals was marked and progressive between 0 and 28 dpi. Thereafter, they tended to stabilize, but remained significantly below control animals until the end of the experiment (Figure 3.1.3C). Two infected animals one from TT-ETBS2 infected group and another from NT-ETBD2 infected group showed severe clinical signs of recumbent and PCV below 15% were euthanized using the above procedure on 30 dpi.

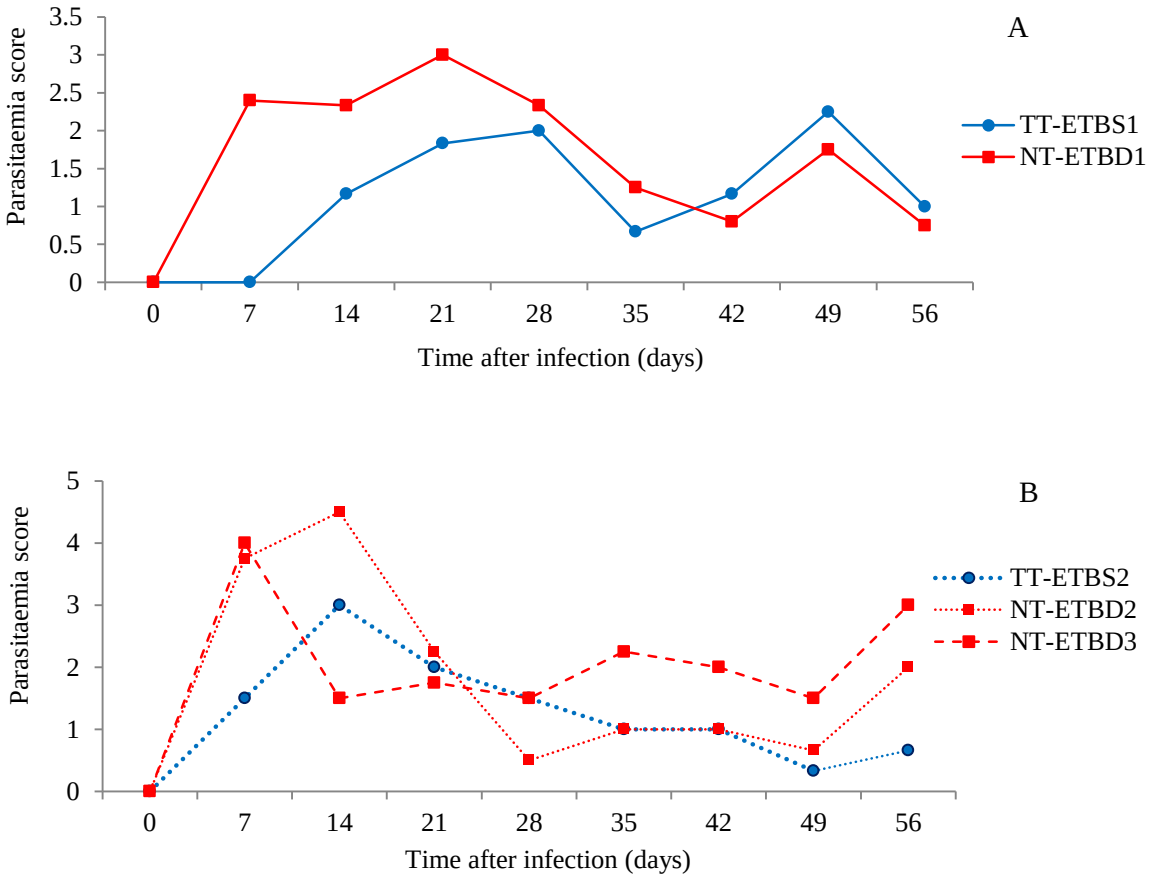


Figure 3.1.1. Mean waves of parasitaemia in score (estimated based on the number of parasites per microscopic field) examined weekly during the study period. (A) Experiment 1- six animals per group *T. vivax* experimentally infected young Zebu cattle (TT-ETBS1: tsetse area isolate 1, and NT-ETBD1: non-tsetse area isolate 1), (B) Experiment 2 - four animals per group *T. vivax* experimentally infected young Zebu cattle (TT- ETBS2: tsetse area isolate 2, NT-ETBD2 and NT- ETBD3: non-tsetse area isolate 2 and 3).

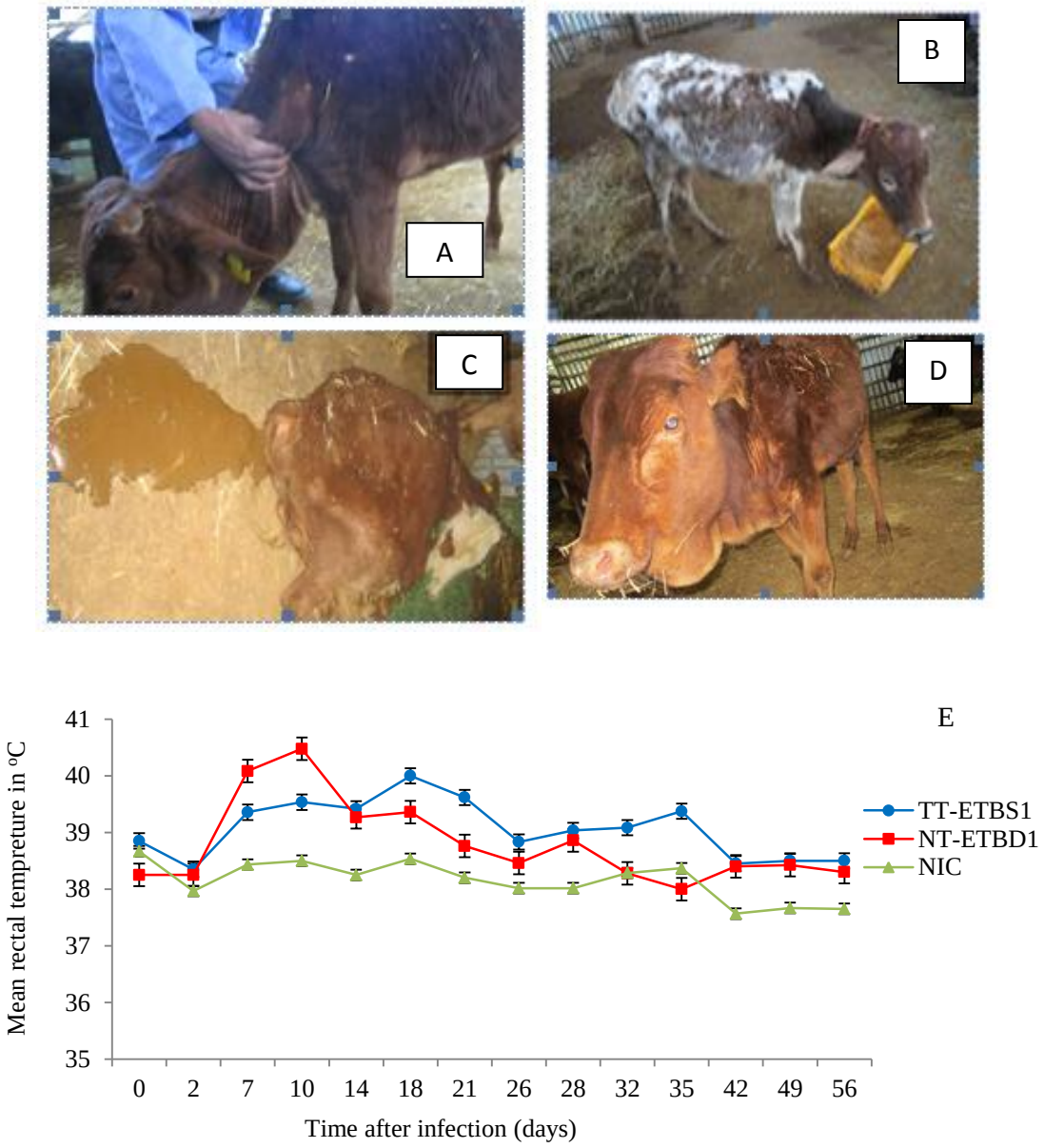


Figure 3.1.2. Major clinical findings in young Zebu cattle - six animals per group experimentally infected with *T. vivax* isolates (TT-ETBS1: tsetse infested area isolate 1, NT- ETBD1: non-tsetse infested area isolate 1 and NIC-non-infected control groups) in experiment 1. (A) Enlarged prescapular lymph node on 12 dpi in TT-ETBS1 infected group, (B) emaciation on 21 dpi in NT-ETBD1 infected group, (C) diarrhea on 12 dpi in NT-ETBD1 infected group, (D) oedema on 19 dpi in TT-ETBS1 infected group, (E) Mean ±SE rectal temperature measured during the study period in TT-ETBS1, NT-ETBD1 and NIC groups.

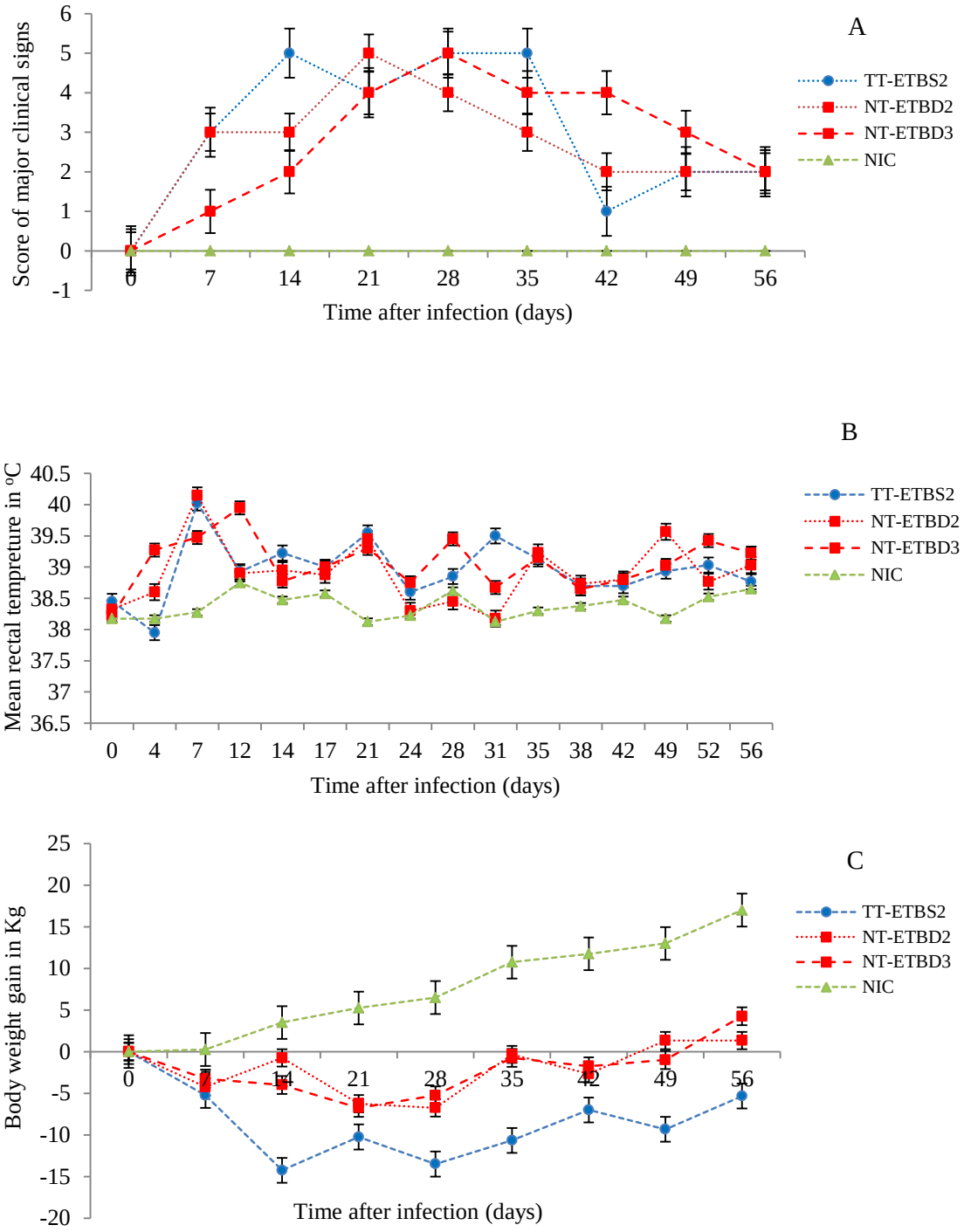


Figure 3.1.3. Clinical findings in young Zebu cattle - four animals per group experimentally infected with *T. vivax* isolates from tsetse-infested (TT-ETBS2) and non-tsetse infested (NT-ETBD2 and NT-ETBD3) areas and non-infected control (NIC) groups during the study period. (A) Score of major clinical signs (reduction in feed intake, enlarged lymph nodes, rough hair coat and pallor of mucus membranes), (B) Mean±SE rectal temperature in °C, (C) Mean ±SE body weight gain in kg.

3.1.3.2. Haematological findings

A significant decrease ($P < 0.001$) in the mean PCV value was detected in both infected groups (23 ±3.6, 20 ±3.7 for TT-ETBS1 and NT-ETBD1 infected groups respectively) compared with the NIC group (27 ±2.1) in experiment 1. Mean PCV values of infected groups was decreased gradually and reached the lowest levels between 42 and 56 dpi (Figure 3.1.4A). In experiment 2 in addition to PCV other haematological parameters were analyzed, and the mean haematological changes of all experimental animals during the study period are summarized in Table 3.1.1.

Table 3.1.1. Mean haematological values during the study period (mean of recordings of 8 consecutive weeks) in young Zebu cattle experimentally infected with *T. vivax* isolates from tsetse infested (TT-ETBS2) and non-tsetse infested (NT-ETBD2 and NT-ETBD3) areas and non-infected control (NIC).

Hematological values	Group	Mean ± SD	95% CI for mean
PCV values (%)	TT-ETBS2	23±3.6^{a}	21.44-24.0
	NT-ETBD2	20±3.7^{ab}	19.08-21.73
	NT-ETBD3	19±4.6^{b}	17.84-20.94
	NIC	27±2.1^{c}	26.50-27.89
Hgb concentration (g/dl)	TT-ETBS2	7.73±1.13^{a}	7.22-8.25
	NT-ETBD2	6.82±1.34^{ab}	6.34-7.30
	NT-ETBD3	6.53±1.86a^{b}	5.90-7.17
	NIC	9.17±0.68^{c}	8.94-9.40
RBC count (x10^{6}/µl)	TT-ETBS2	5.75±0.91^{a}	5.42-6.08
	NT- ETBD2	4.85±1.20^{ab}	4.41-5.28
	NT-ETBD3	4.54±1.46^{b}	4.05-5.04
	NIC	6.47±0.64^{c}	6.25-6.68
MCV (fl)	TT-ETBS2	42.29±3.77^{a}	41.02-43.57
	NT-ETBD2	43.25±7.08^{a}	40.70-45.80
	NT-ETBD2	44.34±7.83^{a}	41.69-47.00
	NIC	39.64±2.98^{b}	38.57-40.72
MCH (pg)	TT-ETBS2	13.46±1.34^{a}	12.98-13.95
	NT-ETBD2	14.40±2.23^{a}	13.60-15.21
	NT-ETBD2	14.72±2.36^{a}	13.92-15.52
	NIC	14.25±1.24^{a}	13.83-14.68
MCHC (g/dl)	TT-ETBS2	33.97±2.36^{a}	33.12-34.82
	NT-ETBD2	33.38±1.61^{a}	32.80-33.96
	NT-ETBD2	33.39±2.56^{a}	32.52-34.26
	NIC	33.74±1.34^{a}	33.28-34.19
WBC count (x10^{3}/µl)	TT-ETBS2	6.78±2.51^{a}	5.87-7.68
	NT-ETBD2	7.04±2.03^{a}	6.31-7.77
	NT-ETBD2	6.75±1.57^{a}	6.21-7.28
	NIC	9.03±1.17^{b}	8.64-9.43

In each parameter of variables, superscripts with different letters indicate significant difference between values at $P < 0.01$.

Consequently, the haematological changes of all experimental animals at different time points of the study period are presented in Figure 1.3.4B-E. A significant decrease ($P < 0.05$) in mean PCV, Hgb, total RBC and WBC counts were observed in all infected groups compared to the non-infected control group. Mean PCV values of infected groups decreased gradually and

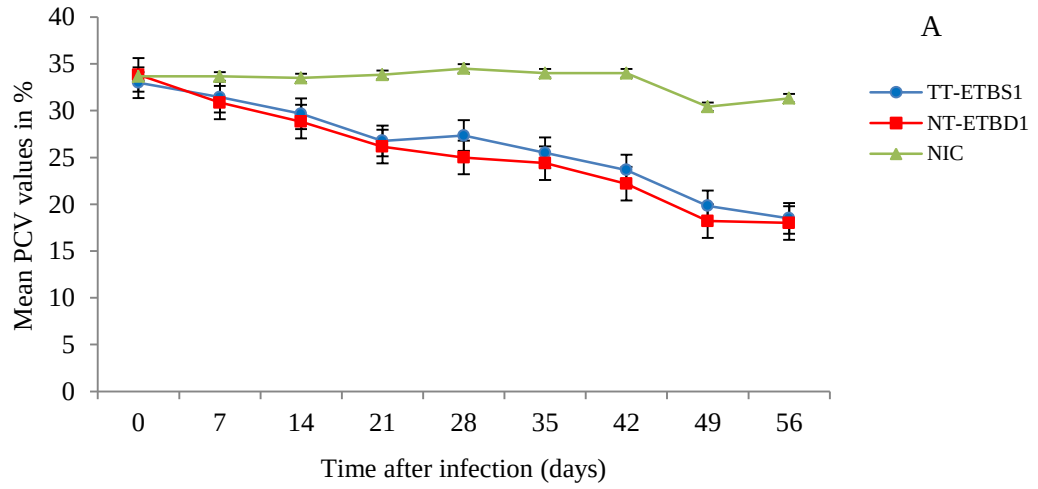
reached significant levels ($P < 0.001$) on 14 dpi (Figure 1.3.4B) compared to non-infected group. In addition, PCV values of group NT-ETBD3 is significantly lower ($P = 0.001$) than that of group TT-ETBS2. Infected groups had a significant reduction ($P < 0.001$) in mean Hgb concentration as compared to the non-infected control group (Figure 1.3.4C). Moreover, mean Hgb concentration value of group NT-ETBD3 is significantly lower ($P = 0.03$) than that of group TT-ETBS2. Reductions in Hgb concentration for groups TT-ETBS2 and NT-ETBD2 started to be significant at day 14 pi whereas for group NT-ETBHD3 started at day 21 which was persistent until the end of the study, but for group NT-ETBD2 it was not significant ($P = 0.062$) on 42 dpi. All of infected groups had a significantly lower ($P < 0.001$) mean total RBC count as compared to control group (Figure 1.3.4D). Total RBC counts of groups NT-ETBD2 and NT-ETBD3 is also significantly lower ($P= 0.007$, and $P < 0.001$, respectively) than that of group TT-ETBS2. Reductions in mean total RBC count become significant at day 14 for groups NT-ETBD2 and NT-ETBD3, but at day 21 for group TT-ETBS2, which had persisted until the end of the study. However, it was not significant on 49 and 56 dpi for groups TT-ETBS2 and NT-ETBD2 respectively. There was a significant increase in mean MCV ($P = 0.001$) in infected groups compared to that of the non-infected group. No significant difference was observed in mean MCH and MCHC values ($P = 0.056$ and 0.581, respectively) between infected and non-infected groups. The mean total WBC count of infected groups was also significantly lower ($P < 0.001$) compared to the non-infected control group (Figure 1.3.4E). However, there was no significant difference in mean WBC count between infected groups.

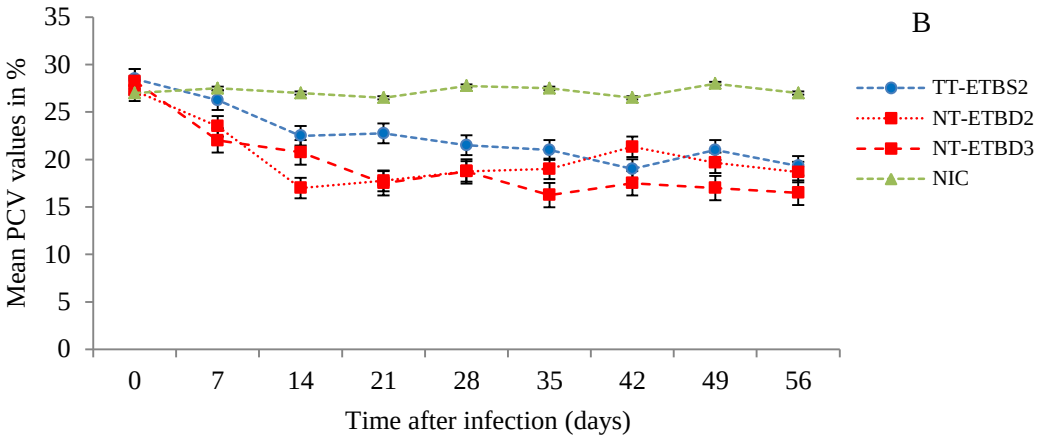
3.1.3.3. Gross pathological findings

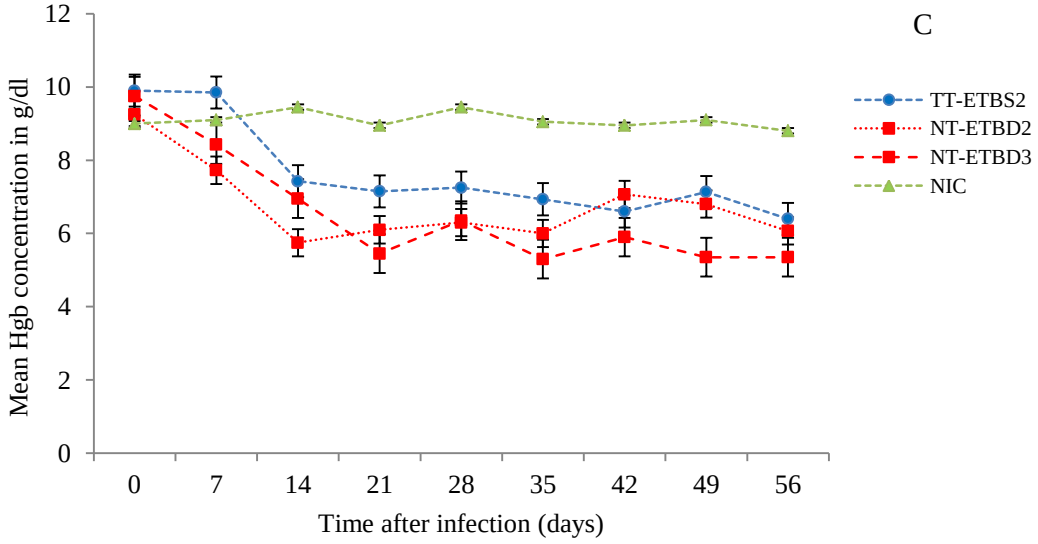
In experiment 1 the most common gross lesions described include splenomegaly with haemorrhagic spleen (Figure 3.1.5A), liver were covered with fibrinous exudates and white spot lesions and enlarged in size (Figure 3.1.5B). Pneumonia with bluish discoloration of the lungs (Figure 3.1.5C), oedema and focal haemorrhages of the brain (Figure 3.1.5D) and enlarged, haemorrhagic and oedematous lymph nodes (Figure 3.1.5E). Other significant and frequent lesions in infected animals were widespread ecchymotic haemorrhages of the small intestine (Figure 3.1.5F), petechial haemorrhage in the kidney and heart. In experiment 2 the most common gross lesions included splenomegaly with haemorrhagic spleen (Figure 3.1.6A), enlarged, haemorrhagic and oedematous lymph nodes (Figure 3.1.6B) and pneumonic lungs (dorso caudal part) especially of interstitial type with rubbery consistency and meaty appearance (Figure 3.1.6C). Liver of most infected animals were covered with fibrinous exudates and were adhered with peritoneum (Figure 3.1.6D). Other significant and frequent lesions in infected animals were widespread ecchymotic haemorrhages on internal organs especially on kidneys (Figure 3.1.6E). Subepicardial ecchymotic and petechial haemorrhages were the common gross lesion on the heart (Figure 3.1.6F). The brain of most infected animals was oedematous and in some there were severe oedema and focal haemorrhages and meningitis (Figure 3.1.6G). However, in both experiments the postmortem examination of the animals in the control group did not reveal any significant gross lesions.

3.1.3.4. Histopathological analysis

In experiment 1 lymph node, spleen, liver and brain tissue were examined for histopathology. The most frequent and common lesions of lymph nodes in infected animals were marked lymphoid hyperplasia with prominent germinal centers. A marked increase of large lymphocytes and plasma cells, and lymphoid depletion with formation of cavity in the lymphoid follicles of cortex was detected (Figure 3.1.7A, B, C). Lymphoid hyperplasia in the white and red pulp of spleen was the common microscopic lesion in spleen of infected cattle. In some animals lymphoid depletion with formation of cavities at the center of lymphoid follicles in the white pulp, and haemosiderosis were detected. The red pulp of the spleen showed lymphoid hyperplasia with formation of follicles dominated by lymphoblasts (Figure 3.1.8A, B, C). The major microscopic changes in the liver of the infected animals were severe zonal hepatic necrosis especially of the centrilobular and periportal regions. Hepatocytes at the center of necrotic areas were either totally lysed or with condensed pyknotic nuclei. A heavy infiltration of mononuclear leukocytes and lymphocytes in the portal areas of the liver was detected. In addition, histopathological examination of the liver revealed dilated blood vessels filled with proteinacious hyaline membranes and fibrin deposits (Figure 3.1.9A B,C). In almost all infected animals, a meningoencephalitis of varying severity was detected. The brain showed neuronal necrosis with shrunken angular neuron and pyknotic somas. In some brains with ischemic type neuronal necrosis and small glial nodules (neuronophagia) were present. Asterogliosis with hypertrophy of astrocytes, perivasculatis with mononuclear cuffs and focal gliosis were also detected in infected brains. In some animals hypertrophy of the vascular endothelium, and an accumulation of a few lymphocytes and macrophages around the affected vessels were present (Figure 3.1.10A, B).


A
Mean PCV values in %
Time after infection (days)
TT-ETBS1
NT-ETBD1
NIC


B
Mean PCV values in %
Time after infection (days)
TT-ETBS2
NT-ETBD2
NT-ETBD3
NIC


C
Mean Hgb concentration in g/dl
Time after infection (days)
TT-ETBS2
NT-ETBD2
NT-ETBD3
NIC

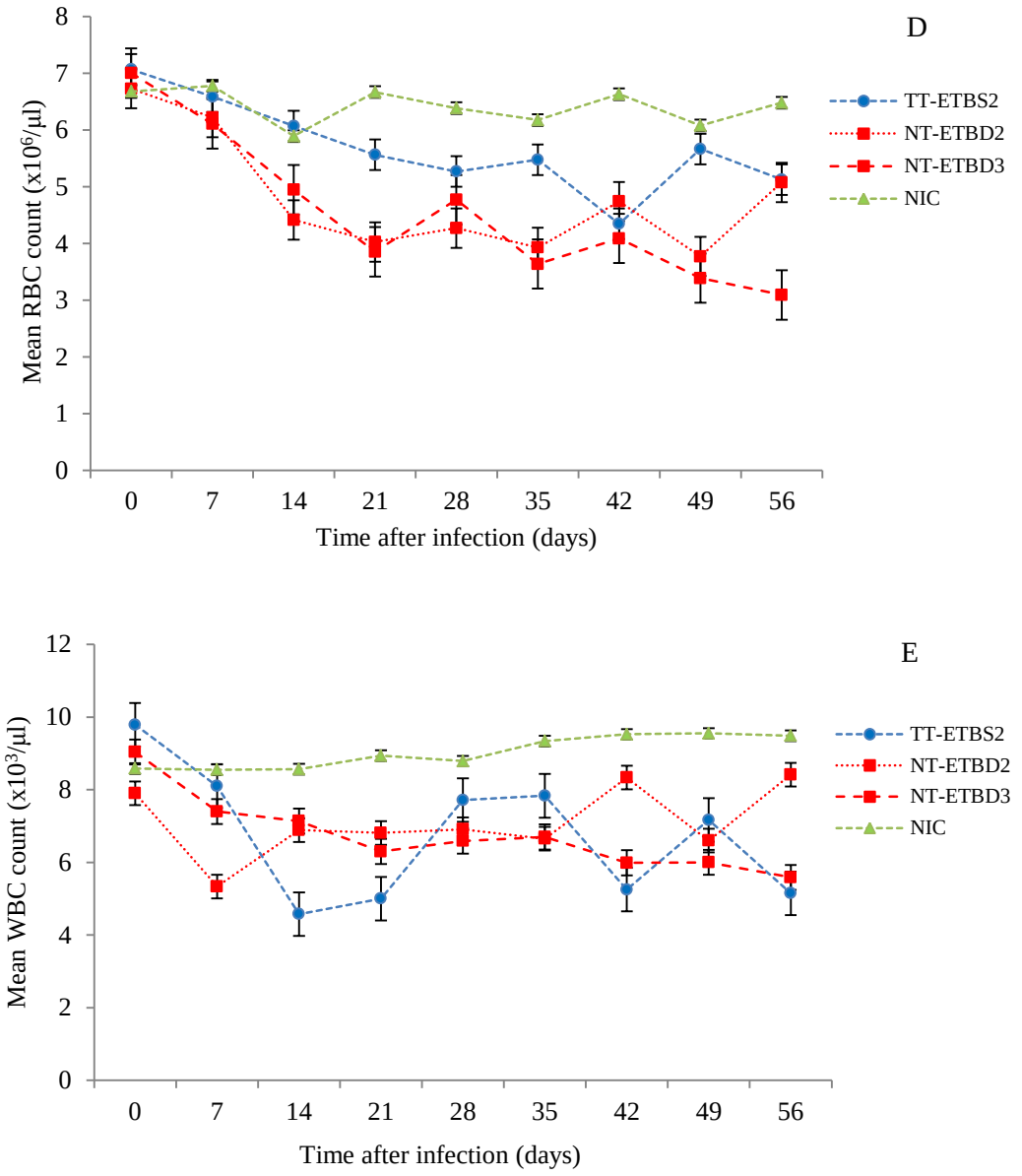


Figure 3.1.4. Mean ±SE haematological values in young Zebu cattle experimentally infected with *T. vivax* isolates (experiment 1 - six animals per group, TT-ETBS1: tsetse infested area isolate 1 and NT-ETBD1: non-tsetse infested area isolate 1 infected groups and NIC-non-infected group and experiment 2- four animals per group, TT-ETBS2: tsetse infested area isolate 2, NT-ETBD2: non-tsetse infested area isolate 2, NT-ETBD3: non-tsetse infested area isolate 3 infected groups and NIC-non-infected group. (A) PCV in percent - experiment 1, (B) PCV in percent - experiment 2, (C) Haemoglobin (Hgb) concentration in g/dl, (D) Total RBC count ($x10^6$cells/µl), (E) Total WBC count ($x10^3$cells/µl).

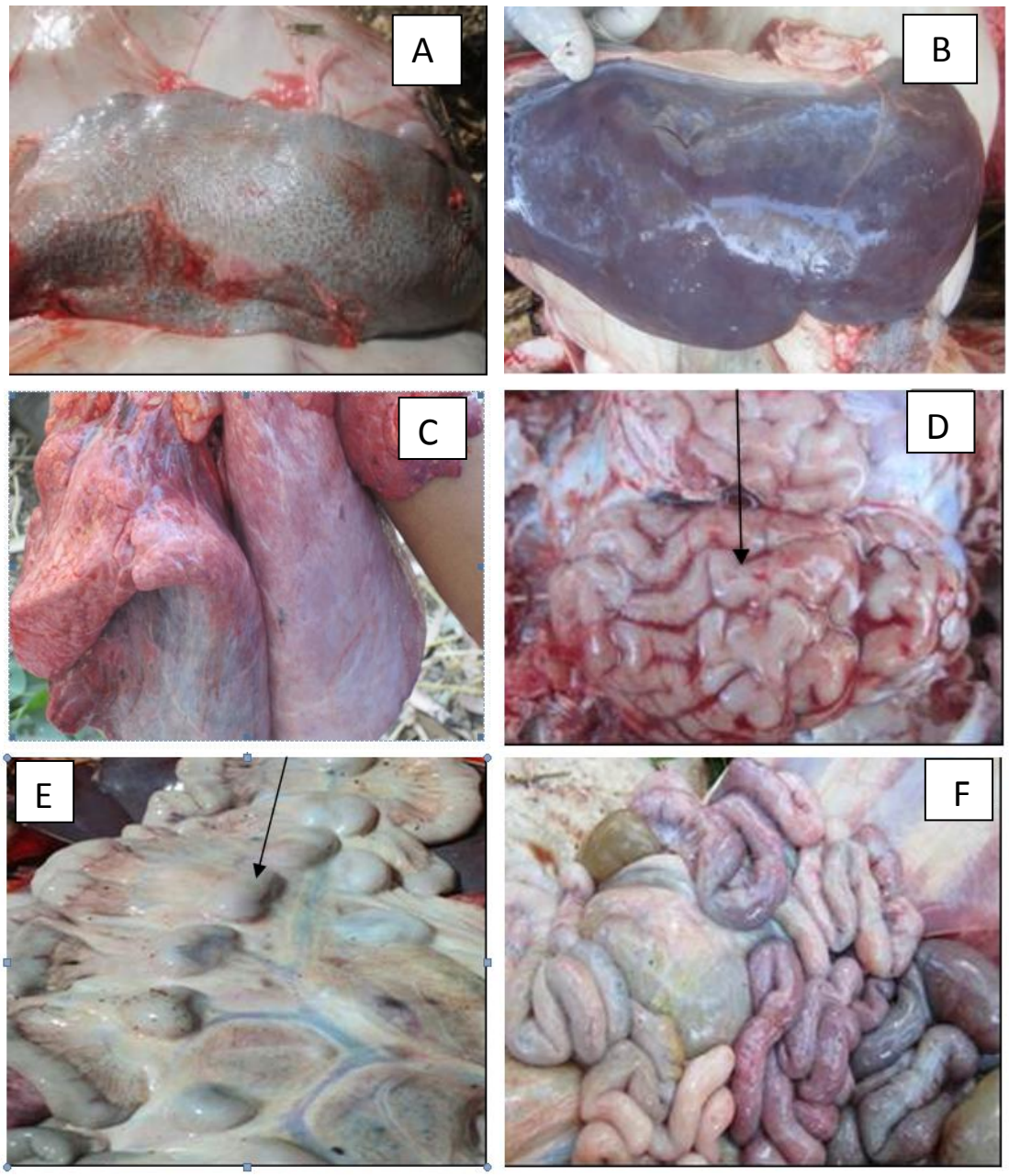


Figure 3.1.5. Major gross pathological findings in young Zebu cattle experimentally infected with *T. vivax* isolates in experiment 1. (A) Haemorrhagic and enlarged spleen with rounded edge, (B) enlarged and oedematous liver with rounded edge and focal necrotic lesions, (C) lung showing emphysema and bluish discoloration on lobular areas, (D) oedematous and haemorrhagic brain, (E) enlarged and oedematous mesentric lymph nodes, (F) haemorrhagic and oedematous intestine.

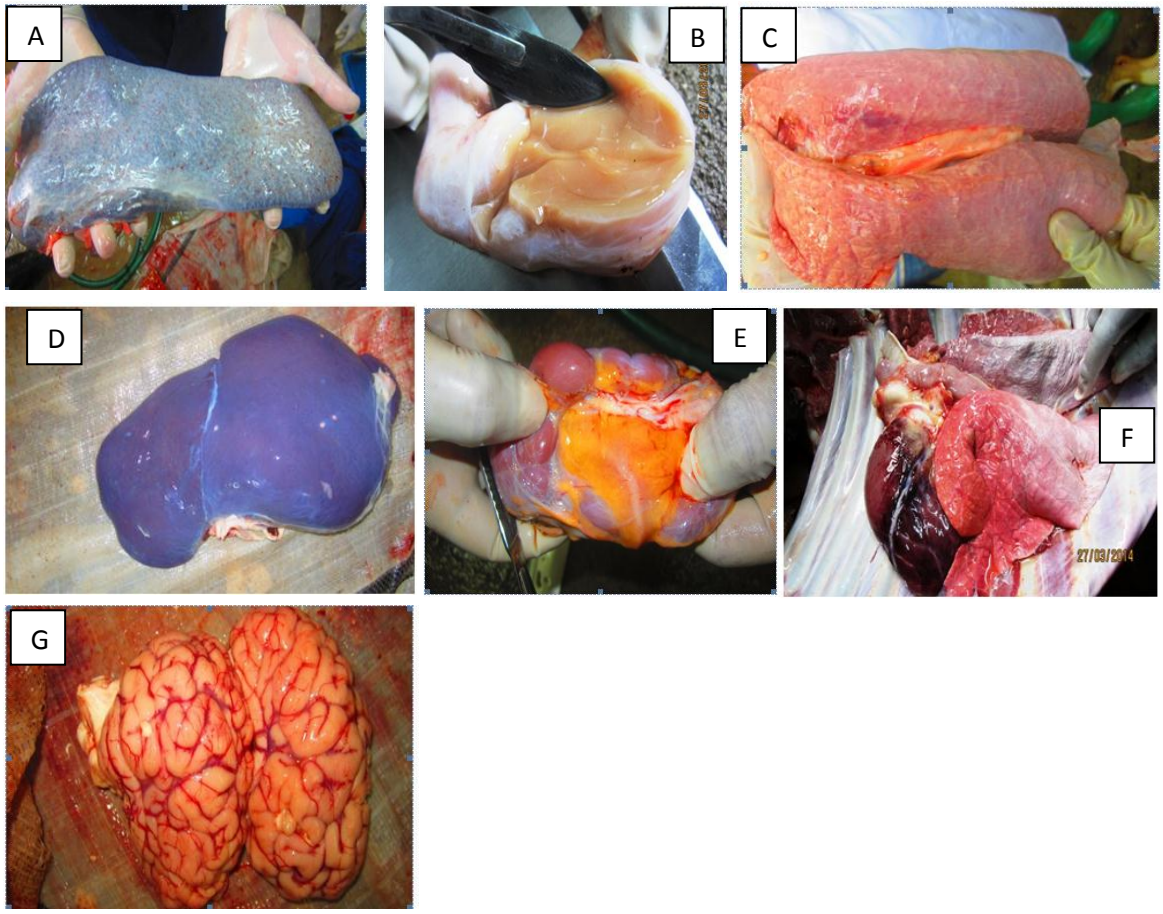


Figure 3.1.6. Major gross pathological findings in young Zebu cattle experimentally infected with *T. vivax* isolates in experiment 2. (A) Haemorrhagic and enlarged spleen with rounded edge, (B) enlarged and oedematous lymphnode, (C) lung showing rubbery, meaty in appearance and distended interstitium, (D) enlarged, and oedematous liver with rounded edge and focal necrotic lesion, (F) swollen haemorrhagic kidney with yellowish discoloration, (E) heart with widespread ecchymotic haemorrhages, (G) oedematous and haemorrhagic brain.

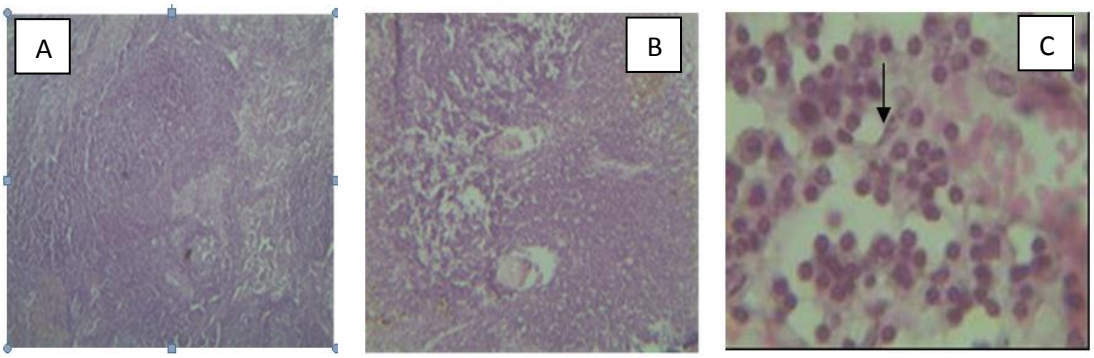


Figure 3.1.7. Major histopathological findings in lymph nodes of young Zebu cattle experimentally infected with *T. vivax* isolates in experiment 1. (A) Enlargement of lymphatic follicles, (B) Severe lymphoid hyperplasia which forms lymphoid cords in the medullary region of the lymph node, (C) Severe infiltration of plasma cells and presence of parasites (arrow).

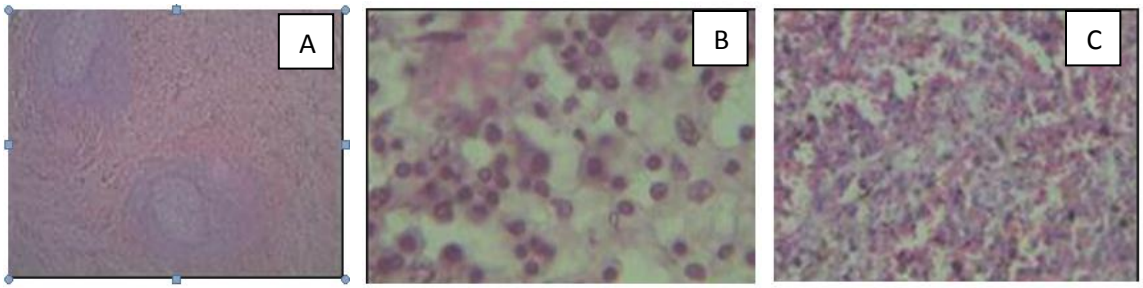


Figure 3.1.8. Major histopathological findings in spleen of young Zebu cattle experimentally infected with *T. vivax* isolates in experiment 1. (A) Haemosiderosis and white pulp with dark zone, (B) Erythrophagocytosis, (C) Spleen with severe lymphoid proliferation forming lymphoid follicles in the red zone.

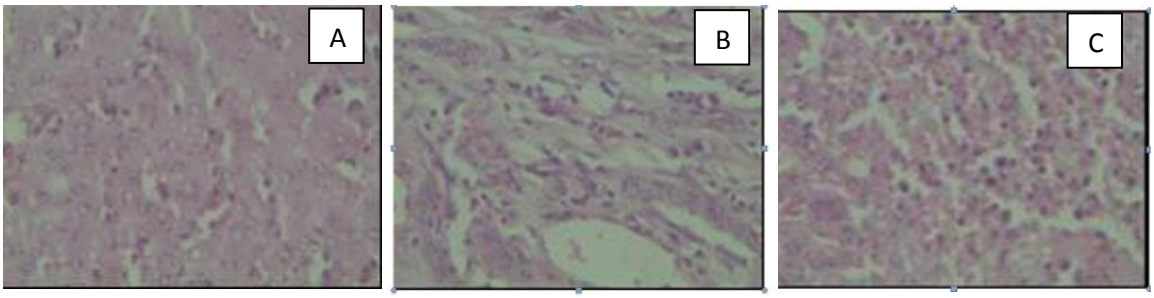


Figure 3.1.9. Major histopathological findings in liver of young Zebu cattle experimentally infected with *T. vivax* in experiment 1. (A) Necrosis of hepatocytes, portal tract and bile duct proliferation, plasma cell and haemorrhage, (B) liver showing centrilobular hepatocyte necrosis, (C) hepatitis with dense accumulation of mononuclear leukocytes especially lymphocytes and plasma cells, proliferation of the biliary ducts and dilation of the vasculatures.

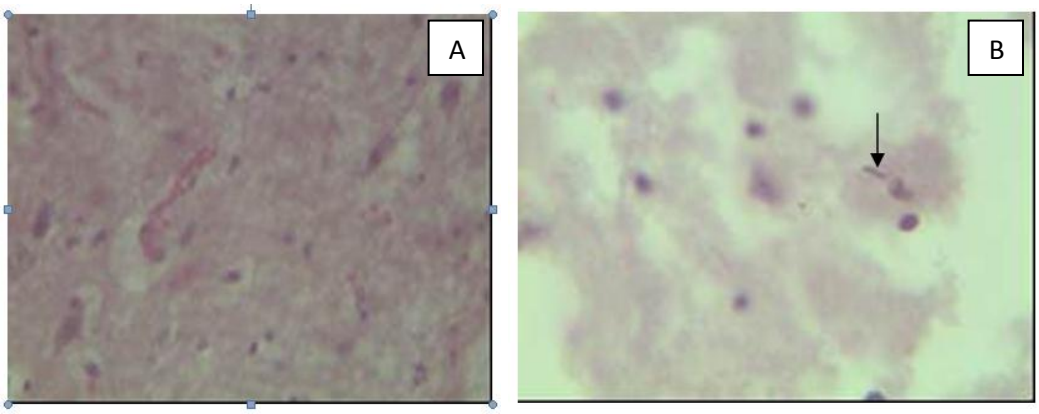


Figure 3.1.10. Major histopathological findings in the brain of young Zebu cattle experimentally infected with *T. vivax* isolates in experiment 1. (A) Haemorrhage and congestion with neuronal necrosis which is characterized by angular and shrunken cell bodies, increased gliosis and small glial nodules, (B) oedema of brain with infiltration of plasma cells and the presence of parasites (arrow).

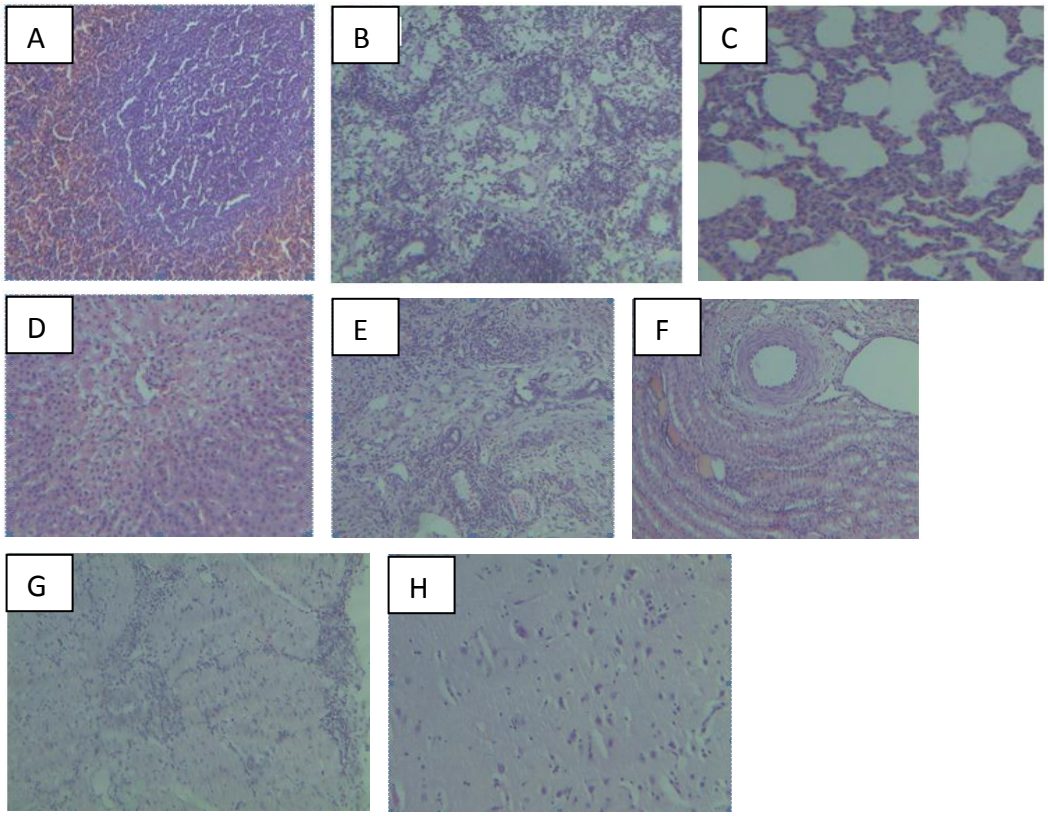


Figure 3.1.11. Major histopathological findings in young Zebu cattle experimentally infected with *T. vivax* isolates in experiment 2. (A) Spleen with severe lymphoid proliferation forming lymphoid follicles in the red zone, (B) lymph node showing severe lymphoid hyperplasia which forms lymphoid cords in the medullary region of the lymph node, (C) lung with severe proliferative interstitial pneumonia with severe compression of the alveoli lumens in affected part, (D) liver showing centrilobular hepatocyte necrosis, (E) cholangiohepatitis with dense accumulation of mononuclear leukocytes especially lymphocytes, plasma cells, macrophages, proliferation of the biliary ducts and dilation of the vasculatures, (F) kidney showing tubulointerstitial nephritis characterized by sloughing of the tubular epithelium, deposition of uniformly staining hyaline substance and infiltration of inflammatory cells into interstitial tissues, (G) myocarditis with heavy infiltration of mononuclear cells especially of lymphocytes, (H) brain with neuronal necrosis which is characterized by angular and shrunken cell bodies, increased gliosis and small glial nodules.

In the second experiment, similarly spleen, lymph node, liver, lung, kidney, heart and brain were analyzed. Lymphoid hyperplasia in the white and red pulp of spleen was the common microscopic lesion in spleen of infected animals. In some animals there was lymphoid depletion with formation of cavity at the center of lymphoid follicles in the white pulp. At the red pulp the spleen showed lymphoid hyperplasia with the formation of follicles dominated by lymphoblasts (Figure 3.1.11A). The most frequent and common lesion of lymph nodes of infected animals were marked lymphoid hyperplasia with prominent germinal centers. There was a marked increase of large lymphocytes and plasma cells (Figure 3.1.11B). In almost all infected animals microscopic examination of the lung showed interstitial pneumonia with severe thickening of interstitial wall due to proliferation of cells in the area (smooth muscle and fibroblasts) which resulted in severe compression of alveolar lumen. The alveoli located at the counter position of those compressed were ruptured resulting in emphysema like lesions (Figure 3.1.11C). The

major microscopic changes in the liver of the infected animals were zonal sever hepatic necrosis especially of the centrilobular and periportal region of the liver. Hepatocytes at the center of necrotized regions were either totally lysed with karyorrhectic nuclei or some are with condensed pyknotic nuclei. There was heavy infiltration of mononuclear leukocytes especially lymphocytes in the portal areas of the liver. In addition, histopathological examination of the liver revealed the blood vessels of the liver are dilated and filled with proteinacious hyaline membranes and fibrin deposits (Figure 3.1.11D, E). The kidneys showed tubulointerstitial nephritis with thickening of the interstitium due to multiplication of fibroblasts and deposition of hyaline membranes in affected tubules (Figure 3.1.11F). The heart showed multifocal myocarditis with infiltration of mononuclear cells especially of lymphocytes, few plasma cells and macrophages. The infection also induced heavy mononuclear cells infiltration to perimysial connective tissue with perivasculatis with domination of lymphocytes. The heart showed myocarditis and myocardial necrosis, sever myocardial degeneration with vacuolization; sever myocardial necrosis with mononuclear cells infiltration (Figure 3.1.11G). In almost all infected animals there is a meningoencephalitis of varying severity in brain. In some brains with neuronal necrosis there were gitter cells, small glial nodules (neuronophagia), ischemic type neuronal necrosis. Lesions in vasculature of the brain were perivasculatis with mononuclear cuffs and focal gliosis. In some animals there were hypertrophy of the vascular endothelium, and an accumulation of a few lymphocytes and macrophages around the affected vessels (Figure 3.1.11H).

3.1.4. Discussion

3.1.4.1. Development of parasitaemia and clinical findings

Trypanosome infections take a variable course depending on factors associated with both the host and the parasites, but are characterized in most instances by the intermittent presence of parasites in the blood and intermittent fever. Three successive stages in infection may occur, namely acute, stabilization and chronic; however, death can occur at any stage. Our epidemiological investigations in Northwest Ethiopia have demonstrated that Trypanosomosis caused by *T. vivax* is prevalent and is causing significant changes indicative of pathological conditions as is reflected by the significant reduction in PCV of infected animals. This has initiated us to look into pathological changes undergoing in the body of infected animals and compare them between those caused by *T. vivax* from tsetse infested and non-tsetse infested areas of the Northwest region of Ethiopia.

In the two experimental studies, infections were established by all *T. vivax* isolates tested and caused clinical trypanosomosis. Regardless of the similar clinical manifestations, the onset of parasitaemia and the appearance of first peak were days earlier in NT *T. vivax* infected groups in both experimental studies. Infection doses are similar for both types of parasites and the experimental animals were grouped randomly, managed similarly and sampling days were identical. Therefore, it is believed that the difference in the onset could be attributed to differences in the genetic basis of the parasites. *T. vivax* isolates from non-tsetse area are better

adapted to mechanical transmission which is almost similar to syringe passage. Further explanation for the early onset of parasitaemia in the NT infected cattle could be the increased growth rate of the NT parasite. This finding is supported by research works on *T. brucei* (Turner et al., 1995) and *P. chabaudi* (Spence et al., 2013) who have shown that when these parasites are syringe passaged in rodents there is a rapid increase in parasitaemia. This has been attributed to a lack of a reset when going through the vector. In the current work the early appearance and peak parasitaemia in the NT isolates seems not to be related to a higher virulence in the NT compared to TT isolates. Similarly other studies also reported that the prepatent period of infection by *T.* vivax is variable, depending on the host and the parasite isolate (Hoare, 1972; Stephan, 1986; Kadima et al., 2000). Our result roughly agrees with the findings of Adeiza et al. (2008) who reported a mean of 5.3 days pre-patent period for *T. vivax* infected goats. However, Osman et al. (2008) reported pre-patent periods of 4.20 ±1.64 and 4.33 ±2.31 days for *T. vivax* infected Nubian and Nilotic dwarf goats respectively. The pre-patent period of infection by *T. vivax* is variable, depending on the immune status of the host, virulence of the parasite isolate and the infective dose (Taylor and Authié, 2004; Osorio et al., 2008).

Clinical manifestations including reduced feed intake, fever, enlarged lymph nodes, oedema, pallor, emaciation and nervous signs were observed in the early stage of the infection in cattle for both types of *T. vivax* and in both experimental infection. Moreover, a total of six animals became recumbent of which four showing nervous signs and all developed PCV below 15%. They were humanely euthanized using an overdose of phenobarbital sodium intravenous administration on 30 days post-infection (dpi). This may suggest the development of encephalopathy associated with hypoglycemia discussed elsewhere in this document and or damage to brain tissue by the parasite (Batista et al., 2011). Altogether, the clinical manifestations suggest that the animals were seriously sick as a result of the infection by *T. vivax* parasites and pathological processes are progressing internally. These have been consistently noticed in both experimental studies. Similar descriptions of clinical signs were reported in several previous studies (Taylor and Authié, 2004; Adeiza et al., 2008). We conclude that irrespective of the geographical location of the parasites, the possible difference in the means of transmission and possible existence of genetic dissimilarity, the two different origins of *T. vivax* isolates have caused similar clinical manifestations. The commencement of pyrexia coincided with the beginning of parasitaemia with similar progression over time. Similar findings were reported by Adeiza et al. (2008) in Savannah brown goats experimentally infected with *T. brucei* and *T. vivax*. In experiment 2 the more pronounced loss in body weight gain of the group in infected with *T. vivax* from tsetse area might be due to the more severe dehydration and anorexia manifested. Furthermore loss of body weight during trypanosome infections in domestic animals is frequently reported by several authors (Rowlands et al., 1994; Osaerio et al., 1998) indicating the economic impact of the disease. The majority of infected cattle in our study developed oedema in the early and late stage of infection particularly in the first experiment might be linked to this hypoalbuminemia. Hopoalbuminemia is a common finding in both experiments (section 3.2). A similar manifestation on oedema has been reported during the chronic stage of trypanosome infections (Orhue et al., 2005).

3.1.4.2. Haematological analysis

Anaemia is considered as a cardinal sign of trypanosome infections in domestic animals. All animals included in our studies were screened for the presence of other anaemia causing parasites including haemoparsites and internal and external parasites prior to the start of any experiment. Moreover, they were treated with acaricides and anthelmintic to remove any undetected infections during the acclimatization period. Therefore, the observed anaemia in our experimental studies is believed to be caused by the *T. vivax* infection.

Accordingly, significant and consistent decrease in mean PCV, Hgb concentration and total RBC counts were observed in all infected groups compared with the non-infected control groups. The reduction in mean PCV values might have a direct correlation with the decline in total RBC counts. These findings are supported by the observations of Maxie et al. (1979) in which pancytopenia, i.e. anaemia, leukopenia, and thrombocytopenia associated with *T. vivax* and *T. congolense* infections of cattle were described. The pallor observed as a consistent clinical manifestation could be the result of this anaemic condition. A sharp decline in indices of anaemia occurred during the first month of the study when parasitaemia and pyrexia were high suggests the possible role of the huge number of parasites and high body temperature in reducing the number of red cells. Living and dead trypanosomes can produce various forms of active chemical substances which can elicit erythrocyte injury (Naessens et al., 2005). Red cell counts can also be reduced as a result of increased erythrophagocytosis which was reported as an important mechanism leading to anaemia in the pathophysiology of *T. congolense* infection in Zambian goats (Witola and Lovelace, 2001). Several other mechanisms are also reported in various studies (Vickerman and Tetley, 1978; Anosa and Kaneko, 1983; Murray and Dexter, 1988; Mbaya et al., 2012). Concomitant with the reduction in PCV and total RBC, there was a rise in mean corpuscular volume whereas the mean corpuscular hemoglobin concentrations were normal; leading to macrocytic normochromic types of anaemia. This implies that, the function of the haematopoietic system is overstretched and is releasing immature erythrocytes into the circulation as it is already described by other researches on *T. vivax* (Anosa and Isoun, 1980; Gardiner, 1989; Nadia et al., 2012). Comparison of anaemia between tsetse and non-tsetse transmitted *T. vivax* revealed that the non-tsetse transmitted *T. vivax* caused more reductions in PCV and hemoglobin concentration compared to its tsetse transmitted counterpart particularly in the second experiment. Further study is required to investigate the cause of such differences as it possibly relate to the difference in the nature of the parasites (Stephan, 1986; Murray and Dexter, 1988; Spence et al., 2013). Generally, it was clearly shown in the two experimental works that anaemia is a typical sign of infection of cattle with both tsetse adapted and non-tsetse transmitted *T. vivax* isolates collected from the Northwest part of Ethiopia. This corroborates the reduced PCV reported during field investigation in naturally infected cattle of the Northwest region of Ethiopia. In the present study, although body weight loss was lower in TT infected group than NT groups, mainly the NT-ETBD3 group was more severely affected by anaemia indicating the possible existence of inter-isolate variation in causing the disease. Moreover, it was demonstrated in section 3.3 of this thesis that the immunological response of the TT group was higher than NT groups in the second experiment which might demand

excessive energy with expense of more body weight loss. Murray and Dexter (1988) showed that the severity of anaemia which follows trypanosome infection could be related to differences in virulence among trypanosome strains.

Decrease in mean PCV values might be correlated with the decrease in total RBC count. A relative deficiency of blood cells occurs initially due to haemodilution and further exacerbated by haemolytic anaemia. Haemolysis could be caused by mechanical injury to erythrocytes by the lashing action of the powerful locomotory flagella and microtubule-reinforced bodies of the high number of the trypanosomes during parasitaemia (Vickerman and Tetley, 1978). Erythrocyte membrane damage has also been associated with adhesion of erythrocytes and reticulocytes to trypanosome surfaces via sialic acid receptors leading to damages to erythrocyte cell membranes (Anosa and Kaneko, 1983). An increase in body temperature was reported to decrease erythrocyte plasticity and longevity in-vivo (Woodruff et al., 1972). An increase in body temperature also increases the rate of immunochemical reactions thereby initiating lipid peroxidation of erythrocytes (Igbokwe, 1994). Our result is in line with these findings, as sharp decline in PCV occurred during the first month of the study when parasitaemia and pyrexia were high. During this period the huge number of parasites and high body temperature may contribute to the severity of anaemia. Furthermore, living and dead trypanosomes can produce various forms of active chemical substances, which can elicit erythrocyte injury (Tizard et al., 1977; Naessens et al., 2005). The reduction in total WBC counts observed in this study was in line with the findings of Maxie et al. (1979), Bengaly et al. (2002) and Allam et al. (2011). The lower counts of WBC observed in the infected groups may be attributed to the immunosuppressive actions of trypanosome infection (Ekanem and Yusuf, 2008).

3.1.4.3. Gross and histopathological analysis

The pathogenesis of tissue lesions varies with the species of trypanosome. *T. congolense* and *T. vivax* are mainly intravascular parasites; they induce changes in the endothelium of capillaries, and so indirectly cause damage to adjacent tissues. The severity of endothelial injury also depends on the interaction of host and parasite (Connor and Van Den Bossche, 2005). An important feature of the pathogenesis of trypanosomosis is the effect on lymphoid tissue, as the disease progresses, the volume of tissue in the spleen, lymph nodes and bone marrow increases markedly. This hyperplasia of reticuloendothelial cells reduces lymphoid cell density, and eventual lymphoid depletion can occur (Taylor and Authie, 2004).

In the current study significant gross lesions and histopathological changes were observed in different organs in *T. vivax* infected animals whereas no notable changes were seen in non-infected control groups. Similar pathological findings were reported by several authors in animal trypanosome infections (Abebe et al., 1993; Batistita et al., 2007; Fatihu et al., 2008; Opara and Fagbemi, 2010; Batistita et al., 2011; Archivio et al., 2012; Silva et al., 2013). In accordance to our finding splenomegaly and hepatomegaly were reported by Fatihu et al. (2008) in experimentally infected goats with *T. vivax*. The various forms of congestion and necrosis observed were also in concordance with findings of other researchers (Archivio et al., 2012;

Silva et al., 2013). Similarly, the perivascular mononuclear cell infiltration, mainly of lymphocytes, plasma cells and macrophages in the lymph node and spleen, and hepatocyte degeneration and hyperplasia in the liver observed in this study agrees with the findings described by Omotainse and Anosa (2009) in ovine infected with *T. vivax*, *T. congolense* and *T. brucei*.

The fact that some trypanosome parasites have been detected in tissue sections such as the brain and lymph node may suggest the possible invasion of the organs by the parasites. However, this should be subject to further study as it could also be possible that they appeared in the tissues during sampling or processing stage. Therefore, the neuronal necrosis and cellular changes noticed in our study might be associated with the presence of parasites in the brain tissue and encephalopathy as result of hypoglycemia arising from reduction of glucose level. This is further corroborated by the fact that four of the infected animals displayed nervous signs suggesting the development of encephalopathy associated with hypoglycemia and or damage to brain tissue by the parasite (Batista et al., 2011). Other studies have also ascribed the nervous system lesions to the presence of trypanosomes in the nervous tissues (Losos and Ikede, 1972; Whitelaw et al., 1988; Tuntasuvan et al., 1997). In the same way study on *T. vivax* infection in Brazilian semiarid region demonstrated that *T. vivax* can cause nervous signs due to inflammatory and degenerative brain lesions in cattle (Batista et al., 2007). In conclusion, despite minor variations in *T. vivax* of non-tsetse infested isolate (NT) caused a more severe anaemia than the tsetse infested *T. vivax* isolate whereas the TT isolate resulted in much higher body weight loss than its NT counter parts and the early onset of parasitaemia observed in NT infected groups in both experiments, tissue damage as reflected by gross and histopathological changes tends to be similar between *T. vivax* parasites of the two ecological zones. Therefore, the *T. vivax* parasite from non-tsetse infested area can at least be considered as important as its counterpart derived from tsetse infested area in causing pathology.

3.2. Comparative biochemical changes in young Zebu (*Bos indicus*) cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia

Adapted from:

Dagnachew, S., Terefe, G., Abebe, G., Barry, D.J., Goddeeris, B.M., 2014. Comparative biochemical changes in young Zebu (*Bos indicus*) cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Veterinary Parasitology **205**, 451-459.

Abstract

The aim of the present study was to determine the effects and compare differences in virulence of *Trypanosoma vivax* infection between tsetse and non-tsetse infested areas of Northwest Ethiopia on the basis of serum biochemical values in young Zebu (*Bos indicus*) cattle. In the first experiment eighteen cattle purchased from a trypanosome free area aged between 9 and 12 months were assigned into three groups of six animals (group TT-ETBS1 = infected with a *T. vivax* isolate 1 from tsetse infested area, group NT-ETBD1 = infected with a *T. vivax* isolate 1 from non-tsetse infested area and group NIC = non-infected control). Similarly, in the second experiment sixteen animals divided into four groups of four animals (group TT-ETBS2 = infected with a *T. vivax* isolate 2 from tsetse infested area, group NT- ETBD2= infected with a *T. vivax* isolate 2 from non-tsetse infested area, group NT-ETBD3 = infected with a *T. vivax* isolate 3 from non-tsetse infested area and group NIC = non-infected control). All experimental animals were kept in a fly proof animal unit and acclimatized for one month prior to the beginning to the experiment. For each experimental animal 2 mL of blood taken from infected donor calves respective of the group was inoculated intravenously at 10^6 trypanosomes/mL except the control group while in the second experiment the control group received 2 mL of non-infected blood from a donor calf. Blood samples were collected once a week for 8 consecutive weeks for the analysis of serum biochemical values (glucose, total cholesterol, total protein, albumin, and enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) using a Humastar 80 clinical chemistry analyzer. In the first experiment a significant reduction ($P < 0.001$) in glucose levels was observed in infected groups compared with the control with mean values of 33.8 ± 3.6 mg/dl for TT-ETBS1, 34.3 ± 3.6 mg/dl for NT-ETBD1 infected groups and 70.9 ± 3.0 mg/dl for control group. A similar reduction was also seen in total cholesterol values ($P = 0.001$) with 70.4 ± 10.6 mg/dl for TT-ETBS1 and 78.0 ± 10.6 mg/dl for NT-ETBD1 infected groups compared to 139.5 ± 8.7 mg/dl for the control group. No difference was observed for total serum protein between the three groups ($P = 0.260$) whereas the mean albumin level was significantly ($P < 0.001$) decreased (3.5 ± 0.1 g/dl and 2.9 ± 0.1 g/dl in TT-ETBS1 and NT-ETBD1 infected groups respectively) compared to that for the control group (4.5 ± 0.1 g/dl). The reduction of albumin levels was also significantly higher ($P < 0.001$) in NT-ETBD1 than TT-ETBS1 infected groups. On the other hand, infected groups had higher ALP values compared to the control ($P = 0.007$), with a mean value of 538.4 ± 64.4 IU/L, 564.9 ± 64.4 IU/L and 273.2 ± 52.6 IU/L for TT-ETBS1, NT-ETBD1 infected groups and control group, respectively. Correspondingly, in the second experiment biochemical analyses showed a significant decreases ($P < 0.001$) in serum glucose, total cholesterol and albumin levels and a significant increases in AST ($P < 0.05$) and ALT ($P < 0.001$) values in all infected groups following infection as compared to the control group. On the other hand, serum total protein and ALP levels was not significant between infected and control groups. Similarly, there was no significant variation in mean values of albumin, total cholesterol, total protein, ALT and ALP between all infected groups. However, there was significantly higher reduction in serum glucose level in TT-ETBS2 than in NT-ETBD3 group ($P < 0.001$) and a higher elevation in AST level in NT-ETBD2 than in TT-ETBS2 groups ($P < 0.05$). In conclusion, all the *T. vivax* parasites

originating from tsetse and non-tsetse infested areas of Northwest Ethiopia caused significant biochemical changes indicative of pathological responses. However, there were no significant variations between the isolates in initiating these changes despite the difference in the onset of parasitaemia and few exceptions of higher reduction in albumin and glucose in the first and the second experiments for NT-ETBD1 and TT-ETBS2 infected groups respectively.

3.2.1. Introduction

The severity of the Trypanosoma infection is influenced by a number of factors; virulence of the different species of trypanosomes, nutritional status and breed of cattle (Murray et al., 1988; Awobode, 2006). Various disorders have been reported consequent to trypanosome infection in animals (Esievo and Saror, 1991; Logan-Henfrey et al., 1992; Adamu et al., 2007). The disease leads to alterations in serum biochemical parameters like liver enzymes, total protein, glucose and cholesterol (Wellde et al., 1989; Katunguka-Rwakishaya et al., 1992; Katunguka-Rwakishaya, 1996; Herrera et al., 2002). Tissue damage/reaction is also one of the pathological indicators of animal trypanosomosis as evidenced by alterations in the serum enzyme levels. Mainly marked elevations in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) have been observed in pigs, rabbits and rats experimentally infected with *T. brucei* (Oruhe et al., 2005) and *T. congolense* (Egbe-Nwiyi et al., 2003). The effects of the trypanosome infection on serum biochemical parameters can have a paramount importance on the pathophysiology and outcome of the disease. Virulence may vary not only because of the species and strain of the parasite, but also due to the mode of transmission. For example, mechanical transmission of *Plasmodium chabaudi* (Spence et al., 2013) and *T. brucei* (Turner et al., 1995) in mice by needle passaging increases its virulence compared to transmission through the insect vector. In the present study areas, without relevant information on the virulence of trypanosomes, trypanocidal drug treatments are given frequently for the control of the disease (Dagnachew et al., 2005; Cherenet et al., 2006; Sinshaw et al., 2006). Data on the virulence of the parasite adapted for cyclical transmission and the one purely transmitted mechanically is important in evaluating treatment responses and host resistance/tolerance to the infections as well as the distribution of the problem. Therefore, in order to determine the disease effects of *T. vivax* from tsetse (cyclical transmission) and non-tsetse (non-cyclical transmission) areas, studies were conducted by measuring serum biochemical values to evaluate the effects of five *T. vivax* isolates two from tsetse infested and three from non-tsetse areas of Northwest Ethiopia in experimentally infected young Zebu cattle.

3.2.2. Materials and methods

3.2.2.1. Experimental setup

All protocols and procedures practiced in the experimental setup are described above in section 3.1.1.1. In short five different *T. vivax* isolates two from tsetse infested area (TT-ETBS1 and TT-ETBS2) and three from non-tsetse infested area (NT-ETBD1, NT-ETBD2 and NT-

ETBD3) and non-infected control groups were tested for biochemical analysis in two consecutive experiemnts.

3.2.2.2. Blood sample collection

During the experimental study period from November 2012 - January 2013 in the first experiment and from March - May 2014 in the second experiment, blood for serum biochemical analysis was collected once a week for eight consecutive weeks from the jugular vein using plain vacutainer tubes. The samples collected for serum biochemical analysis were left on the bench until clotting and then centrifuged at 3400 rpm for 10 minutes. The serum was carefully decanted into aliquots of 1.5 mL eppendorf tubes and kept at -20ºC until biochemical analysis.

3.2.2.3. Determination of serum biochemical values

A Humastar 80 clinical chemistry autoanalyzer machine was used for the analysis of serum biochemical values (glucose in mg/dl, total cholesterol in mg/dl, total protein in g/dl, albumin g/dl, and enzymes including aspartate aminotransferase (AST/GOT) in IU/L, alanine aminotransferase (ALT/GPT) in IU/L and alkaline phosphatase (ALP), and Human GmbH (Wiesbaden, Germany) standard commercial test kits were used according to manufacturer's instruction. The program was set on the instrument for each tests selected from test menu. Thereafter, the machine was calibrated using calibrator (Autocal). Quality control tests are performed with normal (Humatrol N) and pathological (Humatrol P) samples each day, before running samples for tests. Then, adequate controls and serum samples were placed in sample cup and working reagents was added in reagent bottles and put in order in the instrument. The instrument by itself pipettes programmed sample volume and working reagent and incubates for 5 minutes at 37°C. Finally, absorbance was read from color formation at the appropriate wave length and the results were displayed on the screen. The serum values of total protein and albumin were determined by photometric colorimetric test while serum values of glucose, total cholesterol and enzymes were measured by enzymatic colorimetric test.

3.2.2.4. Statistical analysis

Analysis of the mean of serum biochemical values between treatment groups was done with the Statistical Program for Social Sciences (SPSS version-20) computer program. The General Linear Model repeated measure ANOVA was used for statistical analysis and the level of significance was set at $P < 0.05$.

3.2.3. Results

3.2.3.1. Serum biochemical changes

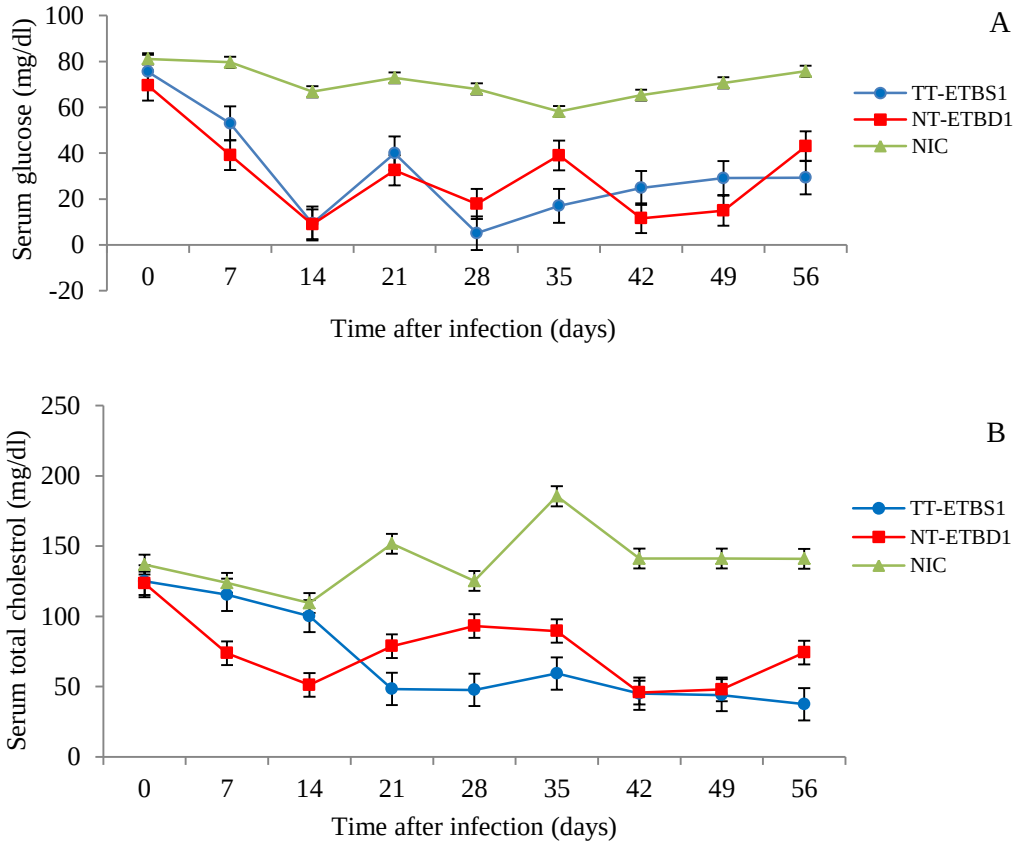
Serum glucose, total cholesterol, total protein, albumin, AST/GOT, ALT/GPT and ALP were analyzed in both experiments during the study period. In the first experiment serum glucose

values of infected cattle ranged from 5.1 to 75.5 mg/dl with a mean value of 33.8 ±3.6 mg/dl, (95%CI: 25.9-41.8) and 8.9-69.5 mg/dl with a mean value of 34.3 ±3.6 mg/dl, (95%CI: 26.3-42.3) in TT-ETBS1 and NT-ETBD1 cattle respectively. Non-infected control cattle had glucose values ranging between 58.2-81.6 mg/dl with a mean value of 70.9 ±3.0 SE mg/dl, (95%CI: 64.4-77.4) (Figure 3.2.1A). Significant reduction in glucose values were found in infected groups of cattle compared with the control group ($P < 0.001$, 95%CI: 24.5-49.7) during each sampling timepoints of the infection period but there was no significant difference observed between the two infected groups ($P = 0.265$, 95%CI: -14.3-13.3). There was a rapid drop in the glucose level between days 5 and 14 pi, followed by a steady increase up to day 21 pi below the pre-infection level and thereafter reduction. At 42 dpi, there was a slow increment until the end of the experiment but without reaching pre-infection levels. The slight steady increase of the glucose level in the later stages of infection in infected groups was not reached at the level of the pre-infection and/or non-infected control group. Mean serum concentrations of glucose in the non-infected group remained fairly constant throughout the experiment.

The total cholesterol values ranged between 37.5-144.3 mg/dl with a mean of 70.4 ±10.6 mg/dl, (95%CI: 47.0-93.8) and 45.6-123.5 mg/dl, with a mean of 78.0 ±10.6 mg/dl, (95%CI: 54.6-101.4) in TT-ETBS1 and NT-ETBD1 cattle respectively. Non-infected control cattle had total cholesterol values ranging between 109.5 and 185.6 mg/dl with a mean of 139.5 ±8.7 mg/dl, (95%CI: 120.4-158.6) (Figure 3.2.1B). Significant reduction in serum total cholesterol values was found in infected groups of cattle (P=0.001, 95%CI: 28.3-103.9) compared with the control group during each sampling timepoints of the infection period but no significant difference was observed between infected groups (P=0.87, 95%CI: -33.0-48.2). A rapid reduction in total cholesterol level was observed from 7 to 14 dpi in the NT cattle and from 14 to 21 dpi in the TT cattle. Mean serum concentration of total cholesterol in the control group remained fairly constant during the experimental period.

Infected cattle had total protein values that ranged from 5.7 to 11.0 g/dl, with a mean of 7.0 ± 0.4 g/dl, (95%CI: 6.3-7.8) and 5.8 to 9.3 g/dl, with a mean of 6.8 ±0.3 g/dl, (95%CI: 6.2-7.7) in TT-ETBS1 and NT-ETBD1 cattle respectively. Non-infected control cattle had total protein values ranging between 6.3 and 10.3 g/dl, with a mean value of 7.6 ± 0.3 g/dl, (95%CI: 7.0-8.2) (Figure 3.2.1C). Significant differences were not observed in total protein values both between infected and control groups or between infected groups ($P = 0.26$). On the other hand serum albumin concentration for the TT-ETBS1 group revealed values ranging from 3.3 to 5.9 g/dl, with mean values of 3.5 ±0.1 g/dl (95%CI: 3.3-3.8), and 3.5-4.5 g/dl, with mean values of 2.9±0.1 g/dl (95%CI: 2.7-3.2) for the NT-ETBD1 group. Non-infected control cattle had serum albumin values ranging between 4.1 and 7.2 g/dl, with a mean value of 4.5 ±0.1 g/dl (95%CI: 4.3-4.7) (Figure 3.2.1D). There was a significant difference in albumin levels between infected and control groups ($P < 0.001$). Significantly higher reduction was also observed in NT-ETBD1 than TT-ETBS1 infected groups ($P = 0.001$, 95%CI: 0.28-0.98). A rapid decline in albumin level until 14 dpi in NT-ETBD1 cattle, a gradual fall in TT-ETBS1 cattle and no change in non-infected control cattle were observed.

Serum AST/GOT values range from 74.5 to 358.2 IU/L, with a mean value of 208.4 ±24.9 IU/L, (95%CI: 129.4-238.9), for the TT-ETBS1 group, and from 83.2 to 349.9 IU/L, with a mean value of 162.5 ±21.9 IU/L, (95%CI: 96.8-206.3) for NT-ETBD1 cattle. In control cattle, GOT values ranged between 73.2 and 234.3 IU/L, with a mean value of 134.5 ±20.3 IU/L, (95%CI: 89.8-179.2) (Figure 3.2.2A). There was no significant difference in GOT values between infected groups of cattle and the control group (P=0.350), but relatively higher mean values were recorded in the infected groups especially in the later stage of the infection for both infected groups and even earlier in the group receiving the tsetse trypanosomes. Infected cattle had serum ALT/GPT values from 22.8 to 59.3 IU/L, with a mean value of 32.5 ±3.4 IU/L, for TT-ETBS1 group and 25.6 to 50.9 IU/L, with a mean value of 33.3±3.4 IU/L, in NT-ETBD1 infected cattle. Control group GPT values ranged from 27.2 to 38.5 IU/L, with a mean value of 31.5 ±2.8 IU/L (Figure 3.2.2B). No significant differences in GPT values were found between infected and control groups of cattle (P=0.482). Serum ALP values ranged from 321.0 to 671.8 IU/L, with a mean value of 538.4 ±64.4 IU/L (95%CI: 396.7-680.1), for the TT-ETBS1 group, and 230.5-880.8 IU/L, with a mean value of 564.9 ±64.4 IU/L (95%CI: 423.2-706.5), for NT-ETBD1 infected cattle. On the other hand, control cattle had ALP values ranging between 214.1 and 352.7 IU/L, with a mean value of 273.2 ±52.6 IU/L (95%CI: 157.5-388.9) (Figure 3.2.2C). Significant increase in ALP values was found in infected groups of cattle compared with the control group (P = 0.007), in each sampling time points of the study period but no significant differences was observed between the infected groups (P = 0.963).



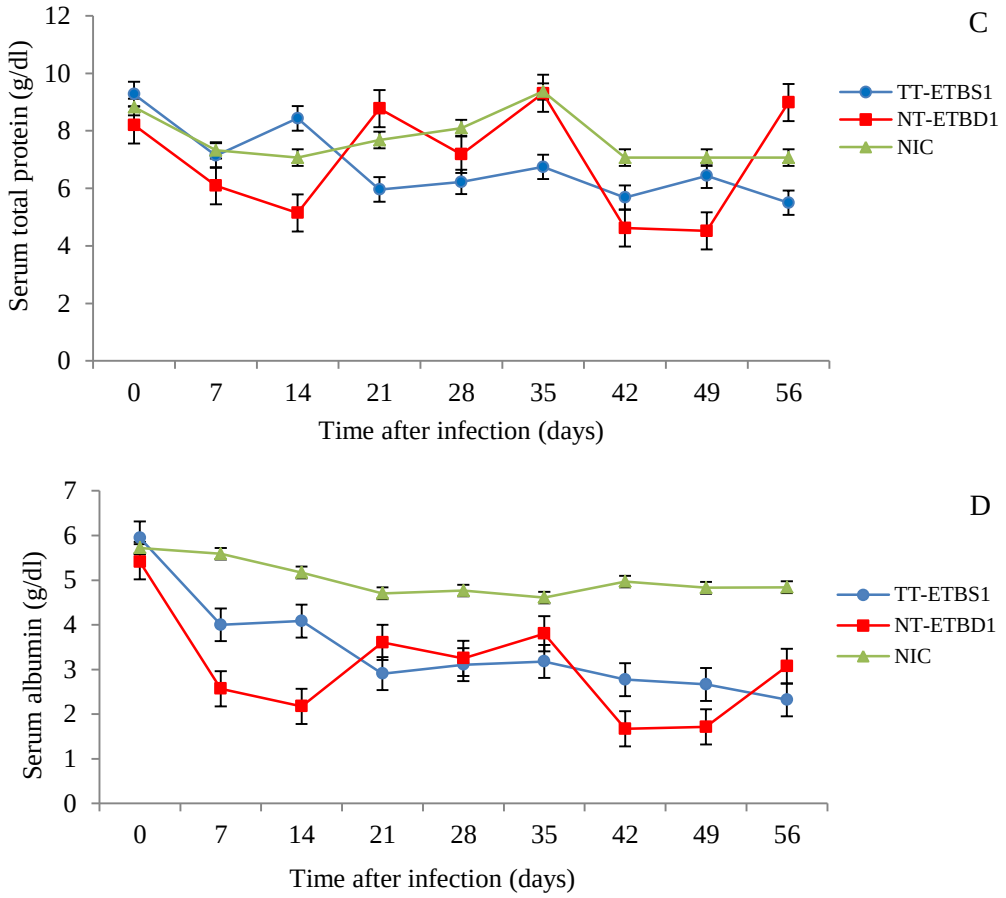


Figure 3.2.1. Mean ±SE serum biochemical changes measured once a week during the study period in *T. vivax* experimental infections of young Zebu cattle - six animals per group (TT-ETBS1:tsetse area isolate 1, NT-ETBD1: non-tsetse area isolate 1 and for NIC-non-infected control groups). (A) Serum glucose concentration in mg/dl, significant difference was found between infected and non-infected groups throughout the infection period. The slight steady increase for the glucose level in the later stages of infection in infected groups was not comparable to non-infected control group, (B) serum total cholesterol concentration, significant difference was found between infected and non-infected groups of cattle throughout the infection period. Significant reduction begins at day 7 pi and the slight steady increase in the later stages of infection in infected groups was not comparable to non-infected control group, (C) serum total protein concentration, (D) serum albumin concentration, significant difference was found between the infected and non-infected control groups throughout the infection period.

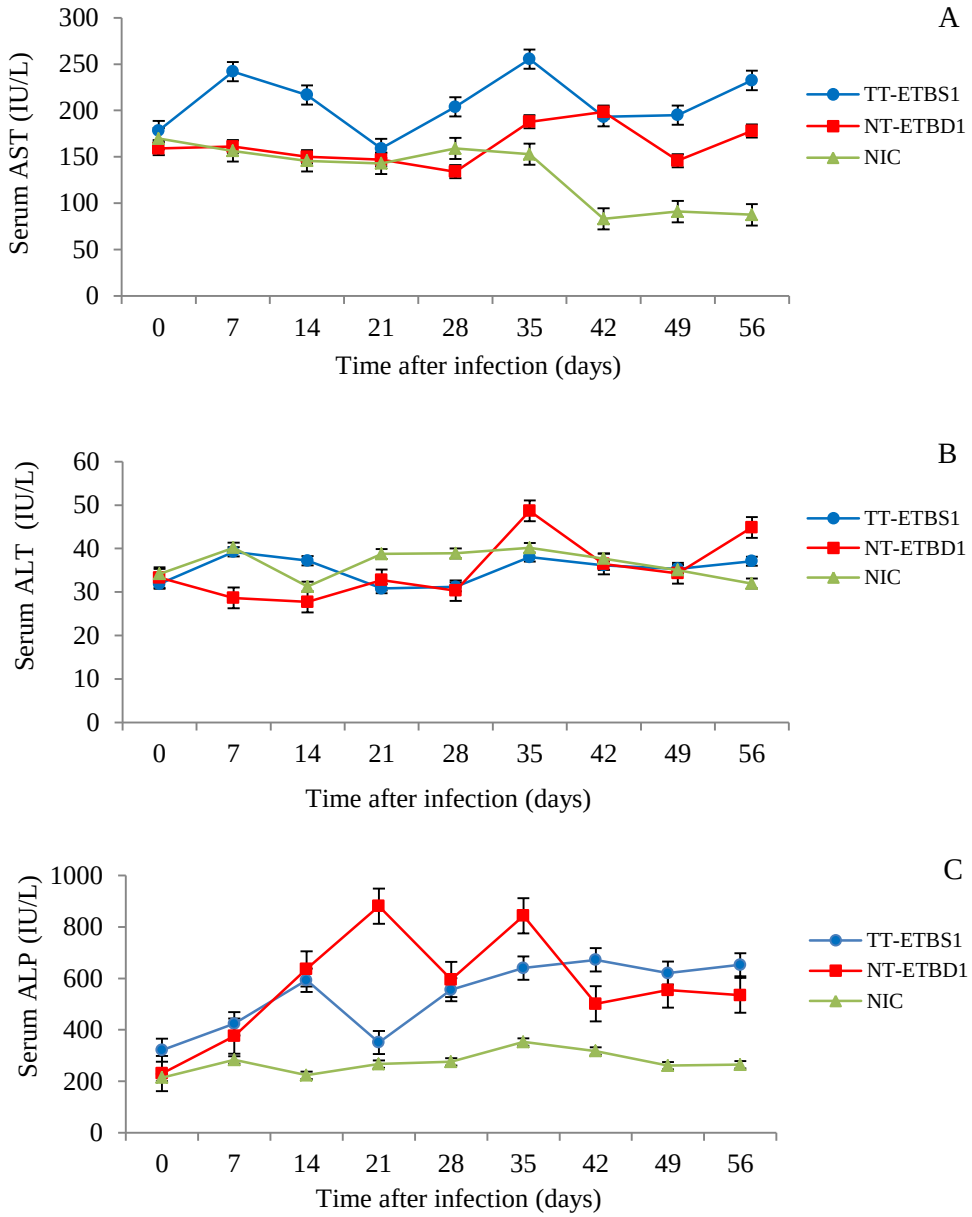
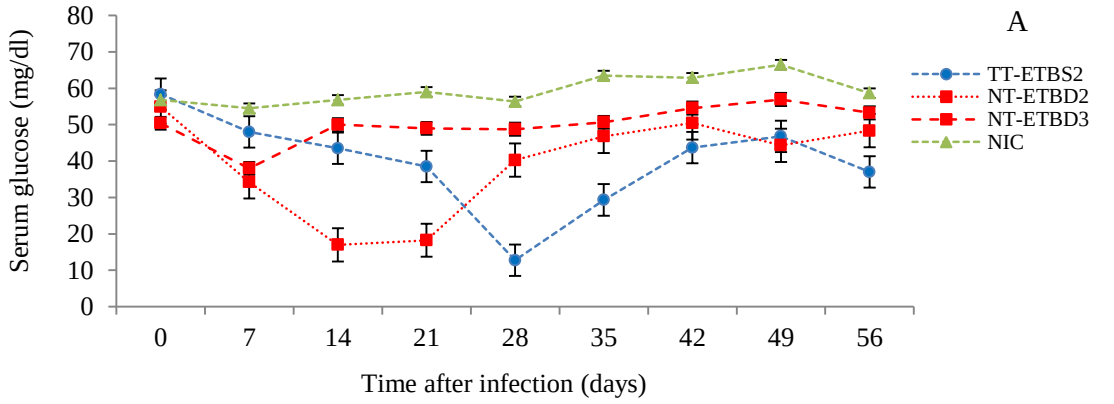


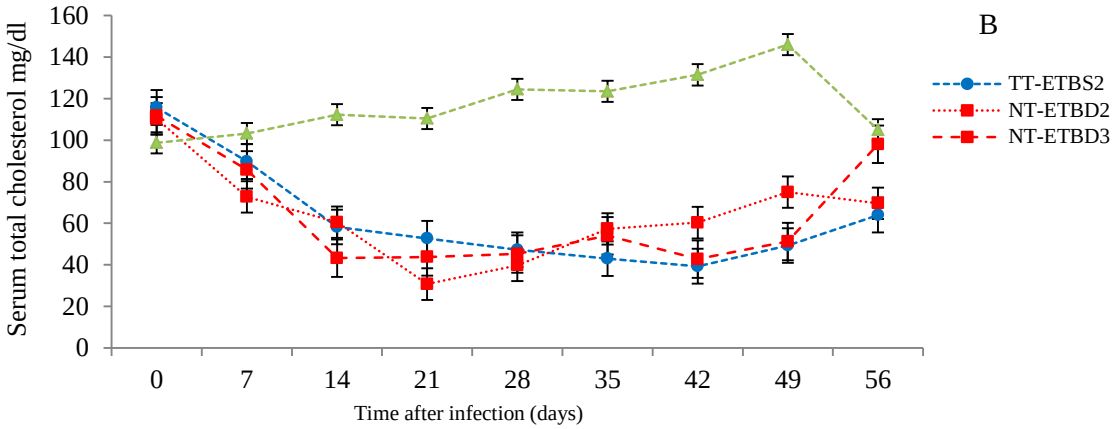
Figure 3.2.2. Mean ±SE serum enzyme changes measured once a week during the study period in *T. vivax* experimental infections in young Zebu cattle - six animals per group (TT-ETBS1: tsetse area isolate 1, NT-ETBD1: non-tsetse area isolate 1 and for NIC-non-infected control groups). (A) Serum GOT/AST concentration in IU/L, (B) serum GPT/ALT concentration in IU/L, (C) serum ALP concentration in IU/L-significant increase was observed between the infected and non-infected control groups beginning from 14 dpi throughout the infection period.

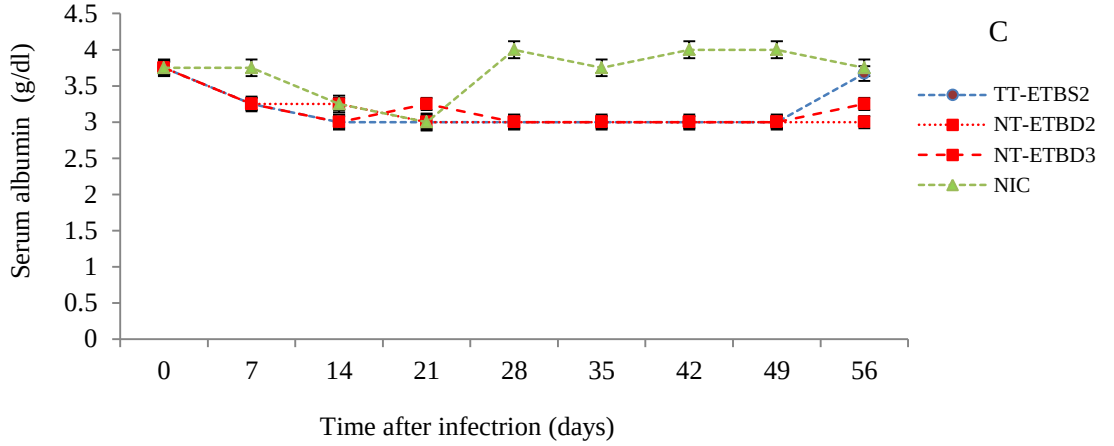
In experiment 2, the mean serum glucose concentration for all infected groups was lower ($P < 0.001$) than that of the non-infected control values (Figure 3.2.3A). Accordingly, groups TT-ETBS2 and NT-ETBD2 have lost glucose until day 21 to 28 pi whereas groups NT-ETBD3 and NIC have maintained their serum glucose levels throughout the experimental period. Moreover, glucose concentrations were lower for group TT-ETBS2 compared to that of group NT-ETBD3 ($P < 0.001$). However, it has no significant variation when compared to group NT-ETBD2. Similarly, the mean serum total cholesterol concentration declined until day 14 (NT-ETBD3), 21 (NT-ETBD2) and 42 (TT-ETBS2) post infection (Figure 3.3.3B). Then there was a gradual increase until the end of the experiment but values still remaining significantly lower than that of control animals ($P < 0.001$). However, the mean total cholesterol concentration between infected groups was not significant ($P > 0.05$).

The mean serum albumin concentration declined from day 0 to day 21 pi for all groups. While control animals which have received non-infected blood have regained their albumin concentration to normality, the values for the infected groups remained significantly lower ($P < 0.001$) until 56 dpi. There was no notable variation in albumin concentration between infected groups all along the experimental period (Figure 3.3.3C). No significant difference was observed in the mean serum total protein concentration between any of the experimental groups ($P > 0.05$). However, a gradual decrease in the mean values of total proteins was observed until day 21-28 pi in the infected animals which later steadily increased to values higher than the pre-infection levels (Figure 3.3.3D).

An increase in serum aspartate aminotransferase (AST) level was observed in NT-ETBD2 and NT-ETBD3 groups 7 dpi. There was significant difference between values of TT-ETBS2 and NT-ETBD2 groups and NT-ETBD2 and NIC ($P < 0.05$). Values for NT-ETBD2 remained higher than those in other groups for the most part of the infection period (Figure 3.3.4A) whereas the other groups including the NIC demonstrated a continuous gradual increase in serum AST level until the end of the experiment and difference was significant only between TT-ETBS2 and NIC from 28 to 56 dpi ($P < 0.01$). The mean serum alanine aminotransferase (ALT) concentration peaked early on day 7 pi for NT-ETBD2 (65.5 IU/L) followed by TT-ETBS2 (65.0IU/L) and NT-ETBD3 (69.25IU/L) values (Figure 3.1.4B). For the most part of the infection period, values for infected groups remained significantly higher than non-infected control ($P < 0.001$). However, the mean ALT concentration between the infected groups was not significant ($P > 0.05$). The mean serum alkaline phosphatase (ALP) concentration first peaked on day 14 pi for NT-ETBD2 (549.75 IU/L) followed by TT-ETBS2 (561.67IU/L) and NT-ETBD3 (502.5IU/L) on day 35 pi, however, for the most part of the infection period, values for infected groups remained comparable to that of control animals ($P > 0.05$). Moreover, there was no significant difference between the infected groups for levels of ALP (Figure 3.3.4C).


A
Serum glucose (mg/dl)
80
70
60
50
40
30
20
10
0
0
7
14
21
28
35
42
49
56
Time after infection (days)
TT-ETBS2
NT-ETBD2
NT-ETBD3
NIC


B
Serum total cholesterol mg/dl
160
140
120
100
80
60
40
20
0
0
7
14
21
28
35
42
49
56
Time after infection (days)
TT-ETBS2
NT-ETBD2
NT-ETBD3


C
Serum albumin (g/dl)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
7
14
21
28
35
42
49
56
Time after infectrion (days)
TT-ETBS2
NT-ETBD2
NT-ETBD3
NIC

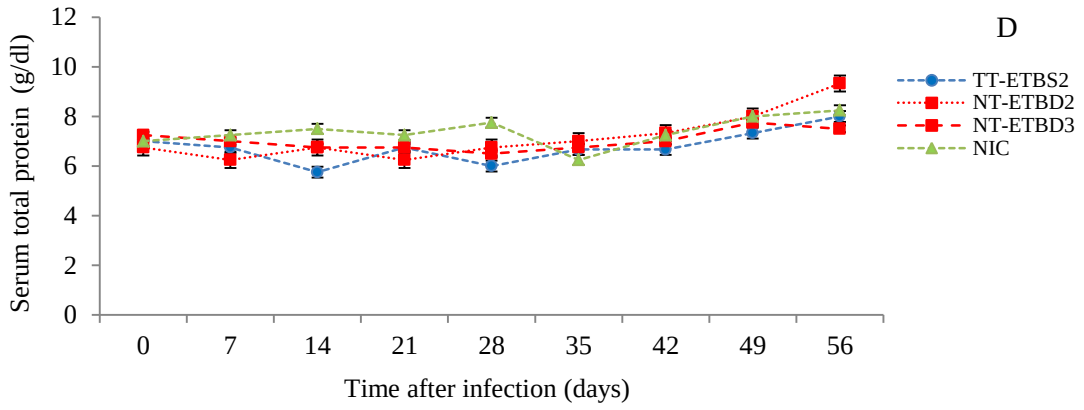


Figure 3.2.3. Mean ±SE serum biochemical changes in young Zebu cattle - four animals per group experimentally infected with *T. vivax* isolates from tsetse infested (TT-ETBS2) and non-tsetse infested (NT-ETBD2, NT-ETBD3) areas and non-infected control (NIC). (A) Glucose, significant difference found between infected and control groups, and between TT-ETBS2 and NT-ETBD3 groups, (B) total cholesterol, (C) albumin, (D) total protein.

3.2.4. Discussion

Parallel to the clinical manifestations and haematological findings, we observed several biochemical changes indicative of pathological and functional disturbances in infected groups. Significant reductions were noticed, following infection, in serum glucose, total cholesterol and albumin whereas change in serum total protein was similar between infected and control groups across both experimental studies. Similarly, there was almost a significant increment in the AST, ALT and ALP levels when considering the two experiments together.

The reduction in the serum glucose levels could be explained by the parasite's need for glucose as an energy source (Opperdoes et al., 1986). Similar reduction in serum glucose levels has been reported in studies in sheep with *T. congolense* (Taiwo et al., 2003), in West African Dwarf goats with *T. congolense* and *T. brucei* (Faye et al., 2005), in cattle with *T. vivax* (Kadima et al., 2000) and in camels with *T. evansi* (Padmaja, 2012). The reduction in the serum glucose levels until 14 dpi could be explained by the parasite's need for glucose as an energy source and the subsequent increases of glucose levels might be when parasites burden reduced seemed to verify the above suggestion of a parasite/glucose relationship and subsequently there occurred alternating fluctuations of glucose level in inverse correlation with the wave of parasitaemia. This type of correlation is a common finding in trypanosomosis (Anosa, 1988). Following trypanosome infection, hypoglycemia was considered important in hyper acute infections characterized by huge numbers of trypanosomes in the circulation (Katunguka-Rwakishaya et al., 1999). Similarly, in the second experiment early reduction in glucose level was pronounced on group TT-ETBS2 and group NT-ETBD2 infected groups compared to group NT-ETBD3 suggesting that changes in glucose concentration following trypanosome infections may vary between strains of the parasite.

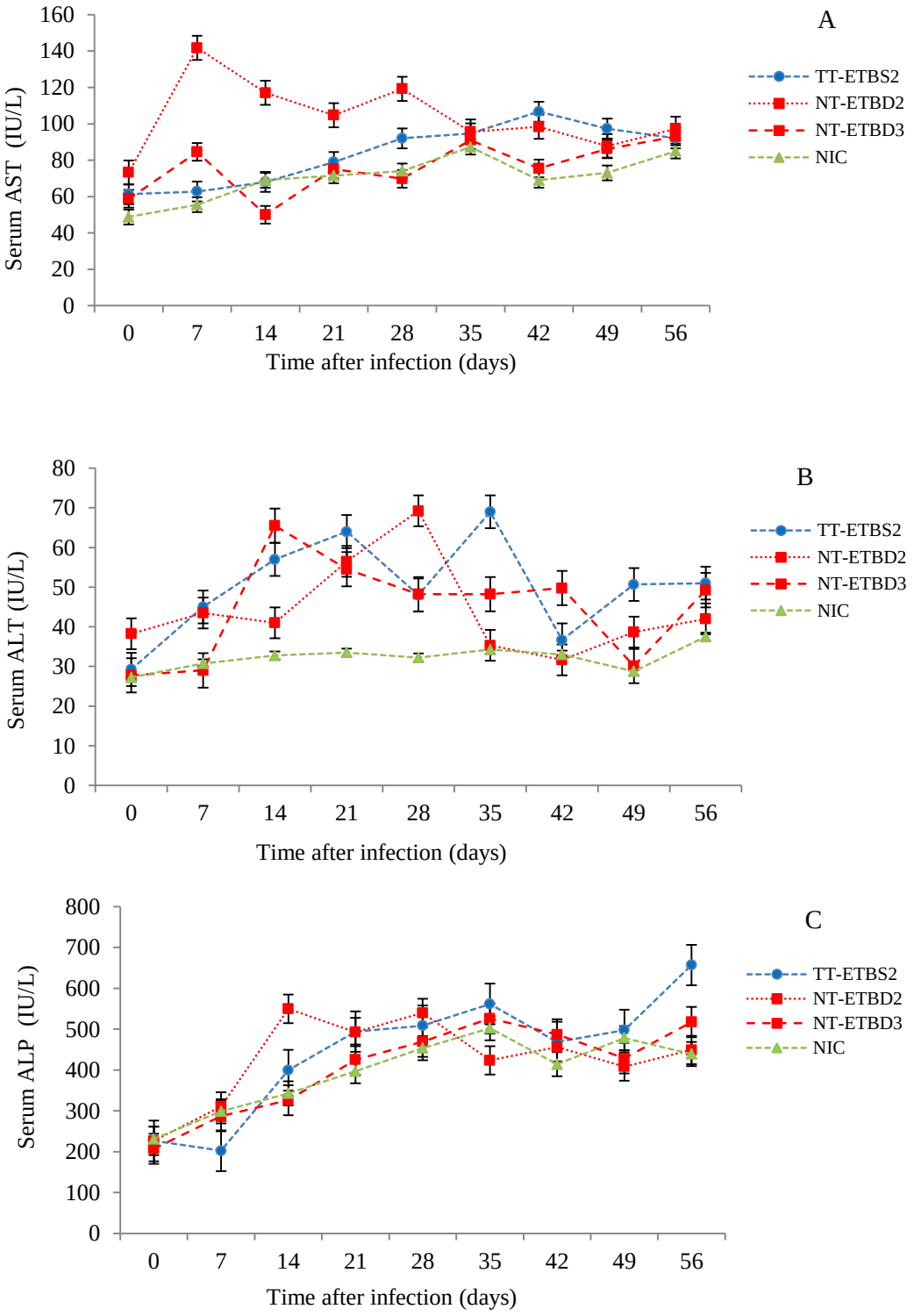


Figure 3.2.4. Mean ±SE serum enzyme changes in young Zebu cattle - four animals per group experimentally infected with *T. vivax* isolates from tsetse (TT-ETBS2) and non-tsetse (NT-ETBD2, NT-ETBD3) infested areas and non-infected control (NIC). (A) Serum AST-significant difference detected between infected and control groups and between NT-ETBD3 and TT-ETBS2 groups, (B) serum ALT significant difference found between infected and control groups, (C) serum ALP.

Four infected animals in the first experiment displayed nervous signs and two infected animals in the second experiment showed recumbence, and all of them resulted PCV below15% were euthanized, which suggests the development of encephalopathy associated with hypoglycemia and or damage to brain tissue by the parasite. Moreover, the findings of nervous signs in the present study could be the possible crossing of brain as indicated in (section 3.1) by *T. vivax*, and similar findings was reported previously in some species of trypanosomes such as *T. brucei*, *T. evansi* and *T. vivax* (Batista et al., 2011). Nervous system lesions caused by these trypanosomes has been associated to the presence of trypanosomes in the nervous tissues (Whitelaw et al., 1988; Tuntasuvan et al., 1997). In the same way study on *T. vivax* infection in Brazilian semiarid region demonstrated that *T. vivax* can cause nervous signs due to inflammatory and degenerative brain lesions in cattle (Batista et al., 2007).

Aberrations of lipid metabolism have been identified in several laboratory and domestic animals infected with various species of trypanosome (Anosa, 1988). The present studies demonstrated that serum total cholesterol level reduces with trypanosome infections in all infected cattle for both experiments compared to non-infected control animals. Similar findings were reported in small East African goats infected with *T. congolense* and *T. brucei* (Biryomumaisho et al., 2003) and in West African Dwarf goats infected with *T. congolense* (Faye et al., 2005). Blood-stream forms of trypanosomes which are unable to synthesize cholesterol, are known to require it along with phospholipids and total lipids for membrane synthesis and growth (Hue et al., 1990; Nok et al., 2003; Adamu et al., 2008). Impaired synthesis which could in turn be the result of insufficient hepatocellular respiration due to hypoxia, caused by anaemia. Reduced subsequent release of cholesterol from the liver could also be a contributory factor to the decrease in serum levels of total cholesterol observed in the trypanosome infected animals (Adamu et al., 2008). Our finding is in accordance with this observation as PCV is significantly reduced in all infected groups which is an indicator for anaemia and subsequent hypoxia.

Reports on serum total protein changes with trypanosome infections show variable findings. The absence of significant changes in serum total protein of the present studies agree with the finding of Faye et al. (2005) in West African dwarf goat infected with *T. congolense* and *T. brucei*. In contrast, Adeiza et al. (2008) reported a decrease in total plasma protein in Savannah brown goats experimentally infected with *T. brucei* and *T. vivax*, suggesting that responses in protein levels may partly depend on animal breed and parasite strain. On the contrary, Yankasa sheep experimentally infected with *T. congolense* demonstrated an increase in serum total protein concentration (Bisalla et al., 2007). Protein levels usually drop in trypanosome infections as a result of depressed albumin levels. The increase in protein levels during the chronic phase of the infection is usually due to the increase in globulin levels, as a result of the immune response by the animals to the infection (Allam et al., 2011). In our findings, despite the lack of significance in total protein changes, reduction in serum albumin level was noted in all trypanosome infected groups compared to control groups. In the first experiment the reduction of albumin level was significantly lower in NT-ETBD1 than TT-ETBS1 infected groups while in the latter experiment no difference between the infected groups. Herrera et al. (2002) showed that total protein and gamma globulins increased while serum albumin decreased

in several trypanosome infections. Nadia et al. (2011) reported similar findings in *T. vivax* infected goats, Faye et al. (2005) in *T. congolense* infected West African Dwarf goats and Osman et al. (2011) in *T. vivax* infected Nubian goats. These changes might be due to liver damage causing depressed albumin values (Cheesbrough, 1999). The majority of infected cattle in our study developed oedema in the early and late stage of infection as explained in (section 3.1) particularly in the first experiment might be linked to this hypoalbuminemia. A similar manifestation on oedema has been reported during the chronic stage of trypanosome infections (Orhue et al., 2005).

Changes in enzyme levels are a good marker of soft tissue damage that results in the alteration of membrane permeability and consequent release of enzymes into the extracellular fluid (Obaleye et al., 2007). In the current studies, we have attempted to evaluate the profiles of ALP, AST and ALT in infected and non-infected groups. When considering the two experiments together, the values of the three enzymes were significantly increased. This clearly shows existence of tissue damage caused by the parasites in these animals. Both pre-infection and control values were lower. Raised activities of ALP as indicated in experiment 1 can be seen in inflammatory conditions of the gut and liver, while active hepatocellular damage is reflected by increases in plasma levels of AST and ALT as mentioned in experiment 2. Similar to our findings (Wurocheke et al., 2008; Oyewole and Malomo, 2009) reported the increased levels of these enzymes in experimental infection of animals with different species of trypanosomes. Losos and Ikede (1972) also reported that the liver has on occasion been regarded as the site of lesions considered characteristic for diseases caused by *T. vivax* and *T. congolense* and the damage will contribute the higher levels of the liver enzymes. The extent of changes in serum biochemical parameters may vary with several factors, including the strain of the parasite and variability in susceptibility to infection which consequently influences the nature and severity of the responses (Anosa, 1988). In this regard, the level of AST was much higher in NT-ETBD2 group than in TT-ETBS2 group in the second experiment whereas there was no difference for ALT and ALP between the two categories.

In general changes in serum biochemical parameters have been associated with several factors (including the strain of the parasite and variability in susceptibility to infection) that influence the nature and severity of the responses (Anosa, 1988). However, this study demonstrated no significant difference between TT and NT infected cattle for all biochemical parameters tested except for serum albumin and glucose where the values were lower in NT-ETBD1 group in the first experiment and in TT-ETBS2 group in the second experiment and higher levels of AST in the NT-ETBD2 than TT-ETBS2 infected groups respectively. This lack in difference is perhaps not surprising as the levels of parasitaemia were high in both infected groups despite their difference in onset. In conclusion, a significant drop in serum glucose, total cholesterol and albumin as well as elevated ALP, AST and ALT levels suggest the presence of pathological conditions following infection with both types of trypanosome origins. Altogether, except for few cases of differences, infection with *T. vivax* derived from both tsetse and non-tsetse areas initiate similar biochemical changes indicative of significant pathology and there is no difference between *T. vivax* parasites originated from the two geographical locations and the

two experimental infections in this respect. Moreover, there was significantly higher reduction in serum albumin in NT-ETBD1 than in TT-ETBS1 roups in the first experiment and in serum glucose in group TT-ETBS2 than in NT-ETBD3 and a higher secretion of AST in NT-ETBD2 than TT-ETBS2 in the second experiment.

3.3. Immune cytokines responses in young Zebu (*Bos indicus*) cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia

Abstract

In Ethiopia, particularly the Northwest region is affected by both tsetse and non-tsetse transmitted trypanosomosis with expected huge impact on livestock productivity. Immune reactions in trypanosomosis do not necessarily lead to protection but appear also to be involved in immunopathology. Therefore, two consecutive experimental studies were undertaken in cattle to analyze their cytokine responses (IFN-γ, TNF-α, IL-10 and IL-12) upon infection with the tsetse adapted or the mechanically transmitted *Trypanosoma vivax* parasites. In the first experiment, eighteen trypanosome naïve Zebu (*Bos indicus*) animals from a trypanosome free area (Debre Brehan: Northcentral highland Ethiopia) were used randomly assigned into three groups of six animals (group TT-ETBS1= infected with a *T. vivax* isolate 1 from tsetse infested area, group NT-ETBD1 = infected with a *T. vivax* isolate 1 from non-tsetse infested area and group NIC = non-infected control). Similarly, in the second experiment, sixteen animals were used. The animals were randomly assigned into four groups of four animals each (group TT-ETBS2= infected with a *T. vivax* isolate 2 from tsetse infested area, group NT-ETBD2= infected with a *T. vivax* isolate 2 from non-tsetse area, group NT-ETBD3= infected with a *T. vivax* isolate 3 from non-tsetse area and group NIC = non-infected control received blood from non-infected donor calf). All animals were kept in a fly proof animal facility and acclimatized for one month prior to the beginning of the experiment. In both experiments, each infected animal in a group received $2x10^6$ trypanosomes intravenously from five different donor calves while animals in the control group of the second experiment inoculated 2 mL of non-infected blood from donor calf. Blood samples were collected once a week for cytokine assays. In the first experiment significant increases were observed for all cytokines ($P < 0.05$) measured in the infected groups compared with the non-infected group, without significant difference between the infected groups except for minor fluctuations in the early stage of the disease where the secretion of cytokines is relatively higher in the NT-ETBD1 than the TT-ETBS1 infected cattle. IFN-γ, TNF-α and IL-12 increased quickly during the first peak parasitaemia and persisted with a steady pattern whereas an increase of IL-10 appears only with the second peak parasitaemia. In the second experiment cytokines (TNF-α, IFN-γ, IL-10 and IL-12) in group TT-ETBS2 showed a significant increase as compared to the control group ($P < 0.05$). However, only the expression of IL-10 in group NT-ETBD2 showed marked increment when compared with the non-infected control values. Significant variation was also observed for IFN-γ, IL-10 and IL-12 between TT-ETBS2 group and the NT-ETBD2 and NT-ETBD3 groups where values for the former are much higher than those for the latter groups. In conclusion, significant differences were demonstrated between infected and non-infected groups of cattle for the immune cytokine responses especially for the TT groups in both experiments suggesting that a profound immunological response has been mounted. However, the lower responsiveness of two groups infected with non-tsetse isolates (NT-ETBD2 and NT-ETBD3) may suggest reduced immunogenicity of the strains. This may have a significant impact on the resistance of the hosts and development of severe clinical disease. Therefore, further study is required to investigate the reason behind such differences with more isoloates and pathogenicity markers.

3.3.1. Introduction

Immune reactions in African animal trypanosomosis do not always lead to protection but appear also involved in immunopathology (Philippe and Bernard, 2006). The genetic background of the host plays an important part in determining disease outcome, either susceptibility or resistance to infection (Kemp et al., 1997; Hanotte et al., 2003). The patterns of cytokine responses during some parasite infections are strongly correlated with the susceptibility of the host (Sher and Coffman, 1992; Clery and Mulcahy, 1998) and the virulence of the parasites could be associated with the surface component VSG which helps the escape from immune reactions, cytokine network dysfunctions and autoantibody production. Both clinical and experimental studies showed high levels of IFN-γ during trypanosome infection (Bancroft et al., 1983; Taylor et al., 1996). The immunoregulatory cytokine IL-10 plays an important role in survival because treatment with anti-IL-10 monoclonal antibody (mAb) causes elevation of serum levels of proinflammatory cytokines and acute death in highly resistant C57BL/6 mice (Shi et al., 2003). The levels of TNF-α, produced by activated macrophages, are correlated with severity of disease and anaemia in trypanosome infections and chronic disease (Naessens et al., 2006). In mice, the early stages of trypanosome infections are characterized by up-regulated synthesis of the Th1 and proinflammatory cytokines, such as IFN-γ, TNF-α, IL6, IL10 and IL12 (Sternberg et al., 2005). Resistance in mice is usually associated with the early production of IFN-γ, which enhances the production of nitric oxide and parasite-specific immunoglobulin G2a antibodies that are important for parasite clearance (Hertz et al., 1998; Magez et al., 2006). Most of the current knowledge on the immune response of African trypanosomosis is derived from murine model on *T. brucei* and *T. congolense*. On top of this, even though high prevalence as well as intensive trypanocidal drug application in both tsetse and non-tsetse infested areas of Northwest Ethiopia (Dagnachew et al., 2005; Cherenet et al., 2006; Sinshaw et al., 2006) was reported, the influence of *T. vivax* infection on the host immune responses has not been analysed. Therefore, the current study was undertaken to analyze and determine differences in the natural hosts' cytokine (IFN-γ, TNF-α, IL-10 and IL-12) responses in experimentally infected young Zebu cattle with two tsetse adapted and three mechanically transmitted *T. vivax* parasites from Northwest Ethiopia.

3.3.2. Materials and methods

3.3.2.1. Experimental setup

All protocols and procedures practiced in the experimental setup are described above in section 3.1.1.1. Briefly five different *T. vivax* isolates two from tsetse infested area (TT-ETBS1 and TT-ETBS2) and three from non-tsetse infested area (NT-ETBD1, NT-ETBD2 and NT-ETBD3) and non-infected control groups were tested for cytokine assays in two consecutive experiemnts.

3.3.2.2. Blood sample collection

During the experimental study period from November 2012 - January 2013 in the first experiment and from March - May 2014 in the second experiment, blood was ccollected using plain vacutainer tubes and hence serum harvesgted and preserved in aliquots for biochemical and cytokine analysis.

3.3.2.3. Serum cytokine assays

The concentration of serum cytokines (IFN-γ, TNF-α, IL-10 and IL-12) secreted during the infection was determined by ELISA kits for bovine cytokines according to the manufacturer's instructions (Bethyl Laboratories, Inc. www.bethyl.com). The sensitivities of the ELISA assays ranged from highest to lowest were determined with standards according to the kits protocol as follows: IFN-γ assay range from 8 - 0.13 ng/mL (8 ng/mL, 4 ng/mL, 2 ng/mL, 1 ng/mL, 0.5 ng/mL, 0.25 ng/mL, 0.13 ng/mL and 0 ng/mL); TNF-α assay range from 5 - 0.078 ng/mL (5 ng/ mL, 2.5 ng/mL, 1.25 ng/ mL, 0.625 ng/ mL, 0.313 ng/mL, 0.156 ng/mL, 0.078 ng/mL and 0 ng/ mL), and IL-10 and IL-12 similar assay range from 1000-15.6 pg/mL (1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL and 0 pg/mL). Avidin conjujated to horseradish peroxidase was added to each microplate well and incubated. After tetramethylbenzidine substrate solution was added, only those wells that contain cytokines, biotin-conjujated antibody and enzyme - conjugated Avidin was exhibited a change in color. The absorbance in color change was measured on an ELISA plate reader at 450 nm.

3.3.2.4. Statistical analysis

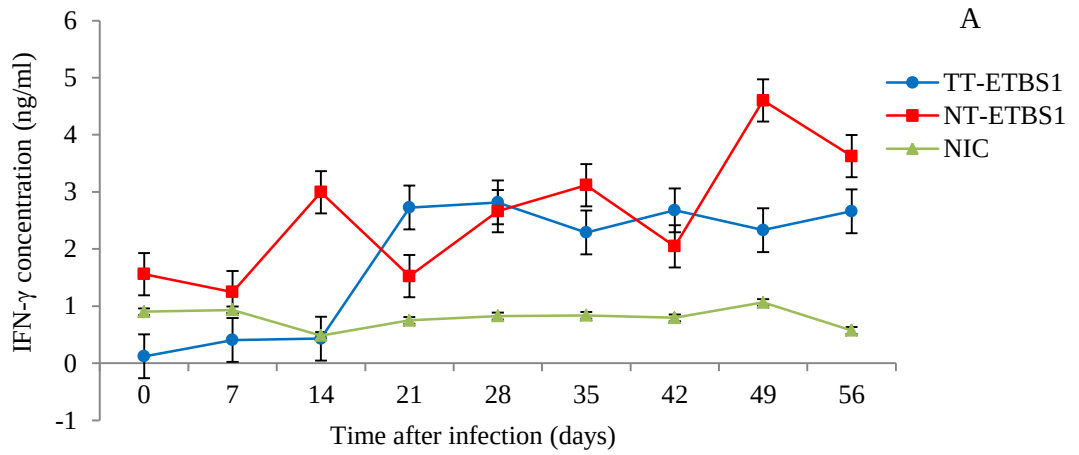
Cytokine concentrations were determined based on a standard curve made with dilutions of the included cytokine standards according to the manufacturer's instructions. GraphPad prism software version 5 was used for the generation of graphs from cytokine data. Differences were considered significant at ($P < 0.05$) using paired sample *t*-test for the concentration of cytokines between infected and non-infected groups as well as between the infected groups at different time points throughout the infection period.

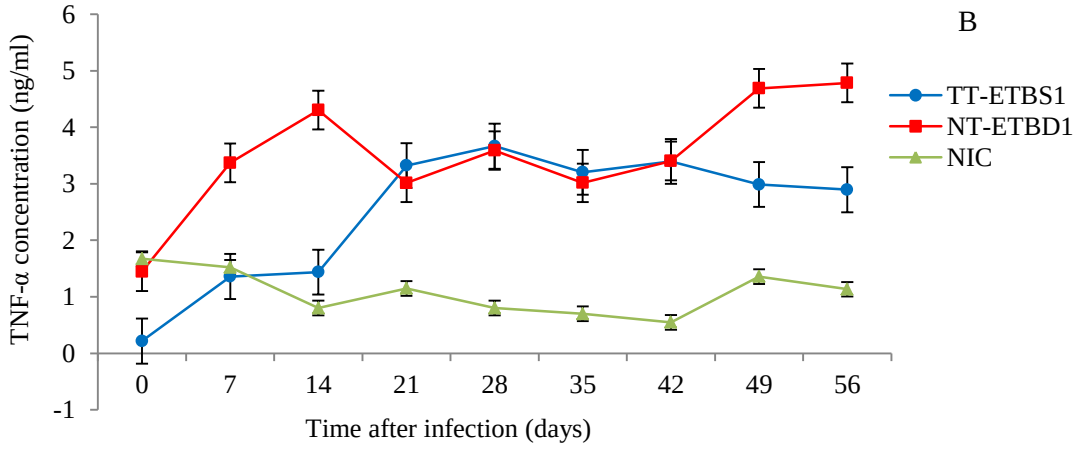
3.3.3. Results

3.3.3.1. Serum cytokine analysis

In the first experiment there was a significant increase ($P < 0.05$) in the concentration of IFN-γ, TNF-α, IL-10 and IL-12 in both infected groups compared with the non-infected control group. However, no significant difference was observed between infected groups for all cytokines values assayed. IFN-γ increased significantly ($P = 0.002$) beginning on 14 dpi in NT-ETBD1 group and on 21 dpi in TT-ETBS1 group ($P = 0.03$) until the end of the experiment (Figure 3.3.1A) compared with NIC. Similar progression was observed for TNF-α with significant increment ($P = 0.003$) beginning on 14 dpi in NT-ETBD1 group and on 21 dpi in TT-ETBS1

group (P = 0.022) throughout the experiment (Figure 3.3.1B) compared with NIC. In the case of IL-12 their concentrations increased significantly before 2 weeks post-infection in group NT-ETBD1 (P= 0.001) corresponding to the early rise in parasitaemic wave but this was delayed until week 2 post-infection for the TT-ETBS1 group (P = 0.007) concomitant with the first parasitaemic wave compared to NIC. Thereafter, the concentration continued to increase in both groups over the whole period (Figure 3.3.1D). The concentration of the regulatory cytokine IL-10 increased significantly after the second waves of parasitaemia in the infected groups (P = 0.003, 0.023, respectively for NT-ETBD1 and TT-ETBS1) compared with values of control animals. The concentration continued to increase during the whole period (Figure 3.3.1C).





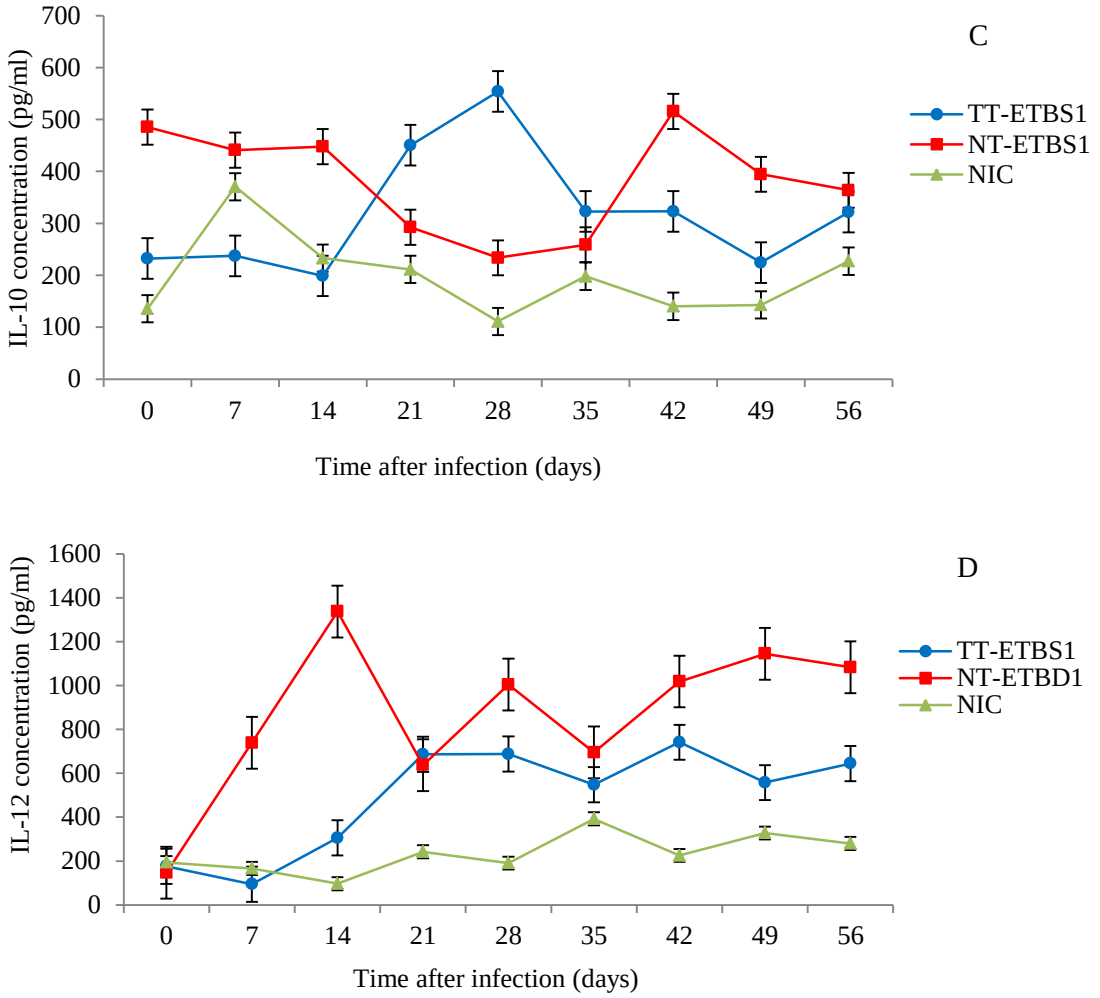
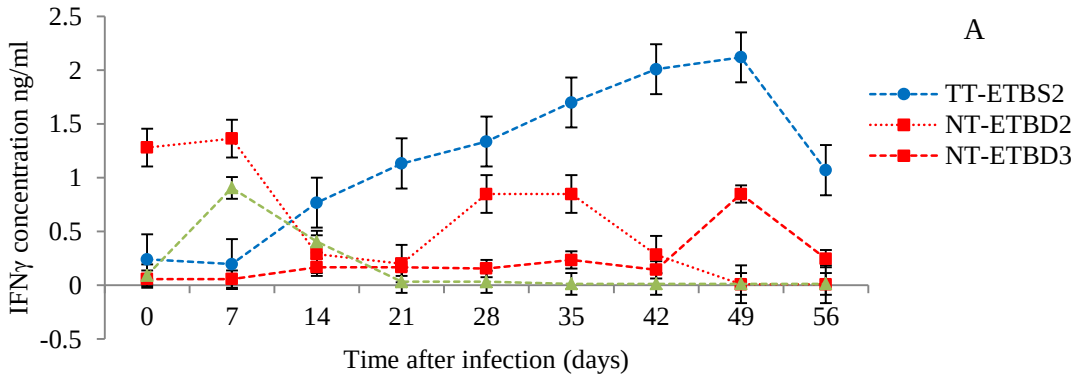
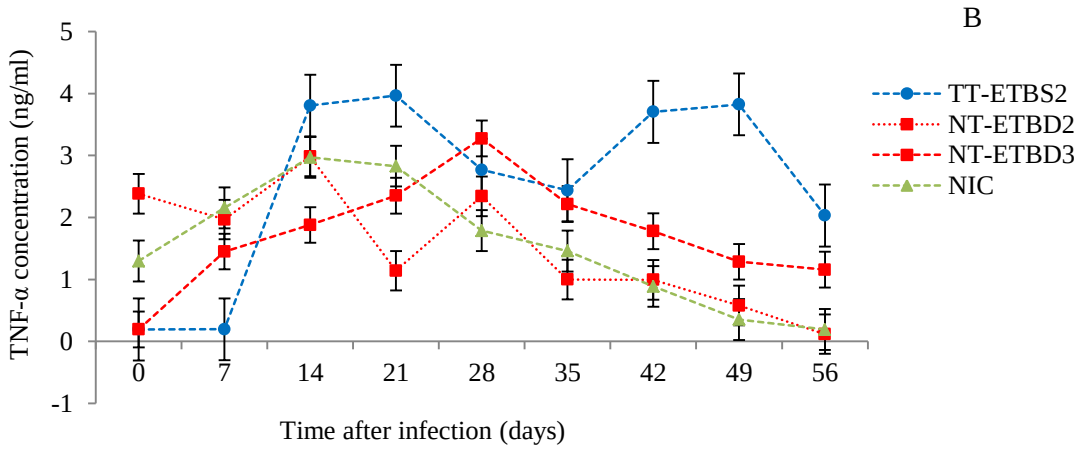


Figure 3.3.1. Mean ±SE serum cytokine concentrations in young Zebu cattle - six animals per group experimentally infected with an isolate of a tsetse-infested (TT-ETBS1) and non-tsetse infested areas (NT-ETBD1) and non-infected control (NIC). (A) IFN-γ, (B) TNF-α, (C) IL-10 and (D) IL-12.

Experiment two demonstrated that a significant increase ($P < 0.05$) in all cytokine values (IFN-γ, TNF-α, IL-10 and IL-12) was observed in TT-ETBS2 infected group while only IL-10 in NT-ETBD2 and none in NT-ETBD3 infected groups compared with the NIC. Similarly, comparison between infected groups indicated that in TT-ETBS2 group significantly ($P < 0.05$) higher secretion of all these cytokines was found than in the NT-ETBD3 group while for NT-ETBD2 only TNF-α. A very high significant increase in the mean concentrations of IFN-γ in group TT-ETBS2 until 49 dpi followed by a sharp decline was observed and the difference from values registered for other groups was remarkable ($P < 0.017$). On the other hand, IFN-γ concentrations for NT-ETBD2 and NT-ETBD3 are comparable to that of NIC group although few animals in each group had demonstrated higher values (Figure 3.3.2A). A significant bimodal peak was observed in TNF-α concentration ($P < 0.05$) for TT-ETBS2 group as compared with the other infected groups and NIC whereas values remained close to control levels for NT-ETBD2 group except on 28 dpi and throughout the infection period for the NT-

ETBD3 (Figure 3.3.2B). Similar to IFN-γ, a very high increase in the mean concentration of IL-10 in group TT-ETBS2 was observed beginning on 14 dpi followed by a decline and the difference from values registered for NT-ETBD3 and NIC groups was significant ($P < 0.05$). The concentration of IL-10 for NT-ETBD2 group was significantly higher ($P = 0.006$) than the NIC group while in NT-ETBD3 group the concentration was comparable to that of NIC group (Figure 3.3.2C). Likewise to IFN-γ and IL-10, a very high increase in the mean concentration of IL-12 in group TT-EBS2 was observed until 35 dpi and the difference from values registered for other groups (NT-ETBD3 and NIC) was significant ($P < 0.05$). However, IL-12 concentrations for NT-ETBD2 and NT-ETBD3 are comparable to that of NIC group although few animals in each group have demonstrated higher values (Figure 3.3.2D).





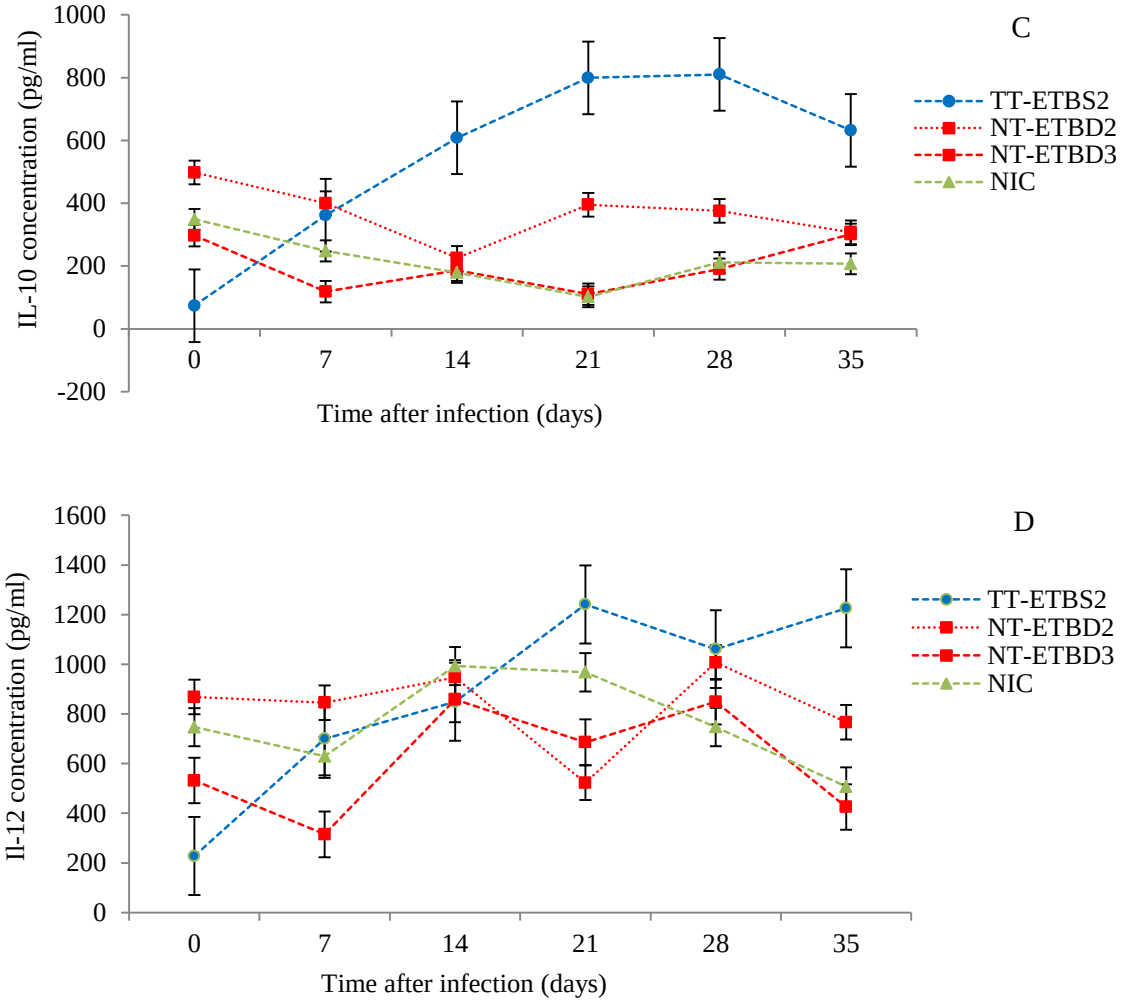


Figure 3.3.2. Mean ±SE serum cytokine concentrations in young Zebu cattle - four animals per group experimentally infected with *T. vivax* isolates from tsetse (TT-ETBS2) and non-tsetse (NT-ETBD2 and NT-ETBD3) infested areas and non-infected control (NIC). (A) TNF-α, (B) IFN-γ, (C) IL-10, (D) IL-12.

3.3.4. Discussion

During the course of African trypanosomosis, a complex interaction between the host immune responses and parasite survival strategies occurs. Surviving a parasitic infection requires the generation of a controlled immune response that recognizes the invading pathogen and that limits a potentially harmful host response. From the point of view of the parasite, microbes have to avoid elimination by the host immune response and sustain their lifecycle while at the same time delay or prevent host destruction. Thus, during parasitic infection, immune regulation can arise as the downstream effect of host response to the parasitic process and/or can be actively induced by the parasite as a survival strategy (Belkaid et al., 2006).

The analysis of serum immune cytokines in the present studies showed a significant elevation in the secretion of IFN-γ, TNF-α, IL-10 and Il-12 in the groups infected by *T. vivax* isolated from

tsetse infested area during both experimental infections. On the other hand, compared to non-infected control values, the cytokine responses against isolates from non-tsetse infested area was significant only for the isolate in the first experiment and only for IL-10 in NT- ETBD2. Relatively we had observed an early increase in the cytokine levels in the NIC group in the second experiment until 21 dpi which was latter reduced and become similar to the first experiment. This variation might be associated to the allogenic immune response of the control group in the early stage of the experiment because of the inoculation of non-infected blood from donor calf. Since the experiments were performed under similar management conditions, this may suggest possible existence of isolate variations to initiate a protective immune response. Concomitant with this observation is that blood parasite load remained low throughout the experimental period in the group TT compared with the NT groups in the second experiment as explained (section 3.1.3).

The later appearance of the immunoregulatory cytokine IL-10 could be attributed to its role as a regulatory or suppressive cytokine probably aiming to reduce immunopathology or could be part of the shift towards Th2 type immune response. It is also possible that the higher levels of IFN-γ secretion could potentially enhance IL-10 secretion, leading to a more pronounced immunosuppression and an enhanced susceptibility as reported in infected BALB/c mice (Shi et al., 2006). IL-10 has also been shown to inhibit IFN-γ and tumor necrosis factor alpha (TNF-α) release by macrophages (Fiorentino et al., 1991; Gazzinelli et al., 1992). Moreover, the occurrence of peak parasitaemic waves was earlier in the NT groups and coincided with earlier secretion of cytokines compared to the TT group in the first experiment (section 3.1.3). This also suggests that the cytokines were induced by the presence of the parasites or their products in the body of infected animals and their concentration is a factor of parasite load.

The increase in TNF-α, IFN-γ, IL-10 and IL-12 recorded in this study largely agrees with the results obtained during infection of cattle and mice by *T. congolense* (Uzonna et al., 1999; Kitani et al., 2004; O'Gorman et al., 2006). The increased secretion of cytokines observed in the present finding is supported by the findings of Yoshihara et al. (2006) who reported IFN-γ activated macrophages increase the immune responses to reduce the number of trypanosome parasites by secreting inflammatory cytokines and by reducing expression level of suppressing cytokine IL-10. For instance, the immunoregulatory cytokine, IL-10 was elevated after the second peak of parasitaemic waves as opposed to the other cytokines which increased at the first peak of parasitaemia. Similarly, an early increase in transcripts for genes encoding proinflammatory mediators (IFN-γ, TNF-α and IL-12) in N'Dama by 14 dpi and by the time of peak parasitaemia, a Th2-like cytokine IL-10 (21dpi) were observed compared to pre-infection levels (Taylor, 1998; O'Gorman et al., 2006). The involvement of the IFN-γ/IL-10 balance in controlling both parasite loads and pathogenicity has also been reported in human African trypanosomiasis (HAT) (Magez and Caljon, 2011). Therefore, our result support the fact that the delayed increment in IL-10 level is to suppress the levels of inflammatory cytokines and consequently reduce both immune competence and immunopathology in the infected groups (Magez and Caljon, 2011). Complementary to this finding is the early appearance of parasitaemia and first peak in the non-tsetse transmitted *T. vivax* infected groups where the

levels of proinflammatory cytokines was low and comparable to control values especially for the two isolates in the second experiment and delayed appearance of IL-10 was observed. Other reports also supports our finding that the analysis of enzyme electrophoretic patterns in 141 isolate of *T. cruzi* extracts from humans and domesticated and wild animals from the endemic zone of Chagas' disease in Argentina confirmed that isolates with different isoezymes differ significantly in their pathogenic activity (Blanco and Montamat, 1998).

In conclusion, significant differences were demonstrated between infected and non-infected groups of cattle for the immune cytokine secretion especially for the TT groups in both experiments suggesting that a profound immunological response has been mounted. However, less responsiveness of two groups against the two non-tsetse isolates may suggest reduced immunogenicity of the parasites. This may have a significant impact on the resistance of the hosts and development of severe clinical disease.

Chapter IV:

Trypanocidal Drug Resistance Tests on *Trypanosoma vivax* Isolates from Tsetse Infested and Non-Tsetse Infested Areas of Northwest Ethiopia with Different Doses of Diminazene aceturate (DA) and Isometamidium chloride (ISM)

Adapted from:

Dagnachew, S., Terefe, G., Abebe, G., Barry, D.J., Richard, M.C., Goddeeris, B.M., 2015. *In vivo* experimental drug resistance study on *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia.
Acta Tropica **146**, 95-100.

Abstract

Ethiopia, particularly in the Northwest region, is affected by both tsetse fly and non-tsetse fly transmitted trypanosomosis with a significant impact on livestock productivity. The control of trypanosomosis in Ethiopia relies on either curative or prophylactic treatment of animals with diminazene aceturate (DA) or isometamidium chloride (ISM), respectively. However, since these two trypanocides have been on the market for more than 40 years, this may have resulted in drug-resistance. Therefore, two consecutive *in vivo* drug resistance tests on five Ethiopian isolates of *Trypanosoma vivax* were completed, two from an area where tsetse flies are present and three from an area where tsetse flies are not present. In the first experiment, two isolates were tested on twenty-four cattle (*Bos indicus*) aged between 6 and 12 months, purchased from a trypanosome-free area (Debre Brehan: Northcentral Ethiopia) and confirmed to be trypanosome-negative, were randomly assigned into four groups of six animals (TT-ETBS1-DA, TT-ETBS1-ISM, NT-ETBD1-DA and TT-ETBD1-ISM), which were infected with *T. vivax* isolated from a tsetse-infested or non-tsetse infested area, and in each case treated with curative doses of DA or ISM. In experiment 2, three isolates one from tsetse and two from non-tsetse area on thirty six animals of the same age and purchased from the same area were divided randomly into six groups of six animals (TT-ETBS2-DA, TT-ETBS2-ISM, NT-ETBD2-DA, NT-ETBD2-ISM, NT-ETBD3-DA and NT-ETBD3-ISM), which were infected with *T. vivax* isolated from a tsetse-infested or non-tsetse infested area, and in each case treated with higher doses of DA or ISM. Each animal was inoculated intravenously with $2x10^6$ trypanosomes from donor animals. In experiment 1, parasitaemia became patent earlier in infections with non-tsetse *T. vivax* (~7 days post-infection) than tsetse (~14 days post-infection) where as in the second experiment it was ~ 4 and ~ 7 dpi, respectively. Both infected groups in the first experiment were treated at the highest peak parasitaemia with DA or ISM consequently nine cattle, five with tsetse *T. vivax* (2 DA- and 3 ISM-treated) and four with non-tsetse *T. vivax* (2 ISM- and 2 DA-treated) showed relapses of parasitaemia. Moreover, treatment did not improve diagnostic host markers of trypanosome infections in these animals. Similarly, in the second experiment, one isolate of non-tsetse infested area against DA in group NT-ETBD2-DA). Three relapsing animals were detected in the group (50% relapsed). Another two relapses were detected one against ISM for the isolate from tsetse infested area (TT-ETBS-ISM) and one against DA for another isolate (NT-ETBD3-DA) from the non-tsetse area. In conclusion, *in vivo* drug resistance tests indicated the presence of resistant parasites (>20% of treated animals in each group relapsed) against recommended doses of both available trypanocidal drugs. Furthermore resistant strains were detected with the higher dose against DA for NT-ETBD2 isolate and suspected resistance problems were detected against ISM and DA for TT-ETBS2 and NT-ETBD3 isolates respectively. Therefore, drug resistance is a threat in both areas and the situation is equally important in non-tsetse area to tsetse infested areas from which the parasites were originated.

4.1. Introduction

African animal trypanosomosis (AAT) is a debilitating and economically costly disease with a major impact on animal health in sub-Saharan Africa. *Trypanosoma vivax*, one of the trypanosome

species responsible for most cattle disease (the other being *T. congolense*), infects a wide host range including cattle, goats, horses and donkeys and is transmitted both cyclically by tsetse flies and mechanically by other biting flies (Hoare, 1972; Swallow, 2002; Mattioli et al., 2004). Ethiopia, particularly the Northwest region, is affected by both tsetse (cyclical) and non-tsetse (mechanical) transmitted trypanosomosis due to *T. vivax* with an associated impact on livestock productivity (Abebe and Jobre, 1996; Abebe, 2005; Sinshaw et al., 2006; Fikru et al., 2012). Trypanosomosis is controlled either by vector control or parasite control, or a combination of both. Parasite control currently relies on a small group of trypanocidal compounds, and new compounds are unlikely to become available in the near future (Barrett et al., 2004). Consequently, the use of these drugs must be carefully monitored and trypanosome populations need to be screened regularly for the appearance of drug-resistant parasites. Several methods have been developed during recent years for the detection of drug resistance in trypanosomes. Most of the *in vivo* and *in vitro* assays used for this purpose are suitable for the determination of drug resistance of a small number of isolates, but are less appropriate for large-scale screening. However, there is increasing evidence that the efficacy of chemotherapy is becoming reduced by the widespread development of trypanosome drug resistance (Van den Bossche, et al., 2000; Delespaux, 2002). Drug resistance to isometamidium chloride (ISM) is more widespread than to diminazene aceturate (DA) (Geerts et al., 2001), but increasingly there are reports of resistance to both drugs (Geerts and Holmes, 1998; Mungube et al., 2012). Recent reports (Sow et al., 2012; Vitouley et al., 2012) confirmed ISM and DA resistance in *T. vivax* and *T. congolense* in West Africa. In Ethiopia the appearance of drug-resistant trypanosomes has been reported by several authors (Mulugeta et al., 1997; Afework et al., 2000; Tewelde et al., 2004; Shimelis et al., 2008; Moti et al., 2012). However, the reports on drug resistance are focused mainly on *T. congolense* and the importance of drug resistance as a problem for *T. vivax* is less well studied. Therefore, the work in this study was undertaken to test and compare the drug resistance at lower and higher doses of *T. vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia.

4.2. Materials and methods

4.2.1. Experimental setup

4.2.1.1. Experimental study site

The experimental studies were carried out from November 2012 - February 2013 and March - June 2014 for the first and second experiments respectively in a fly-proof animal facility located in the premises of the College of Veterinary Medicine and Agriculture of Addis Ababa University at Debre Zeit.

4.2.1.2. Experimental animals

A total of 60 animals 24 for the first experiment and 36 for the second experiment, of indigenous Zebu (*Bos indicus*) cattle aged 9 to 12 months were purchased from a trypanosome free area (Debre Brehan: northcentral Ethiopia) located at 9°4´N and 39°32´E with an altitude of 2840 m.a.s.l.

about 130 km north of Addis Ababa. The animals were transferred into a fly-proof experimental animal house of the College of Veterinary Medicine and Agriculture of Addis Ababa University at Debre Zeit located at 9°6'N and 37°15'E with an altitude of 1920 m.a.s.l. about 47 km east of Addis Ababa. Animals were ear-tagged, examined for the presence of trypanosomes and other blood parasites using blood smear technique (Paries et al., 1982) and faecal egg count method (Soulsby, 1982) for helminthes. To avoid occurrence of pneumonia associated with transport stress and change of environment, each animal was treated on arrival with oxytetracycline 20% w/v (Chongqing Fangtong Animal Pharmaceutical Co., Ltd, China). All animals were treated with albendazole 2500 mg bolus and Ivermectin (Chengdu Qiankum Veterinary Pharmaceuticals Co.Ltd., China) to control internal and external parasites. After treatment prior to the beginning of the experiment animals were acclimatized for one month for the new environment, handling and feeding conditions.

4.2.1.3. Experimental design

The efficacy of recommended curative and higher doses of diminazene aceturate (DA) and isometamidium chloride (ISM) were tested against *T. vivax* isolates in experimentally infected cattle based on previous established protocols (Eisler et al., 2001). For the first experiment animals were randomly assigned into four groups of six animals per group: group TT-ETBS1-DA = infected with *T. vivax* isolate 1 from tsetse infested area and treated with DA; group TT-ETBS1-ISM = infected with *T. vivax* isolate1 from tsetse infested area and treated with ISM; group NT-ETBD1-DA = infected with *T. vivax* isolate 1 from non-tsetse infested area and treated with DA; and group NT-ETBD1-ISM = infected with *T. vivax* isolate 1 from non-tsetse infested area and treated with ISM. Similarly, in the second experiment animals were assigned randomly into six groups of six animals each: group TT-ETBS2-DA = infected with *T. vivax* isolate 2 from tsetse infested area and treated with DA, group TT-ETBS 2-ISM = infected with *T. vivax* isolate 2 from tsetse infested area and treated with ISM, group NT-ETBD2-DA = infected with *T. vivax* isolate 2 from non-tsetse infested area and treated with DA, group NT-ETBD2-ISM = infected with *T. vivax* isolate 2 from non-tsetse area and treated with ISM and group NT-ETBD3-DA = infected with *T. vivax* isolate 3 from non-tsetse infested area and treated with DA and group NT-ETBD3-ISM = infected with *T. vivax* isolate 3 from non-tsetse infested area and treated with ISM.

4.2.1.4. Trypanosome isolates and challenge

Trypanosoma vivax isolates tested for the drug resistance are those *T. vivax* parasites studied for the determination of pathogenicity (TT-ETBS1 and TT-ETBS2-Ethiopia Birsheleko isolate 1and 2, and NT-ETBD1, NT-ETBD2 and NT-ETBD3-Ethiopia Bahir Dar isolate 1, 2 and 3) for tsetse infested and non-tsetse infested areas respectively. Each experimental animal received 2 mL of infected blood from the donor animals at 10^6 trypanosomes/mL via the intravenous route.

4.2.1.5. Trypanocidal drugs

The trypanocidal drugs used in the first experiment were diminazene aceturate (Diminasan™, Lot No. PS012910, Exp. 04-2016, ALFASAN, Kuipersweg 9, Woerden, Holland) and isometamidium chloride (Veridium™, Lot No. 198A1, Exp. 06-2015, CEVA SANTE ANIMALE, Libourne-France). Diminazene aceturate (DA) was injected as a 7% solution at dose of 3.5 mg/kg body weight, and isometamidium chloride (ISM) was injected as a 1% solution at a dose of 0.5 mg/kg of body weight. Similarly in the second experiment diminazene aceturate (BERENIL R.T.U, Lot No.A189A01, Exp.03-2015, 20 Spartan Rd., Spartan, Republic of South Africa) and isometamidium chloride (Veridium T.M, Lot No.198A1, Exp. 06-2015, CEVA SANTE ANIMALE, Libourne-France) were tested. Diminazene aceturate was injected as a 7% solution at dose of 7 mg/kg body weight and isometamidium chloride was injected as 1% solution at dose of 1 mg/kg of body weight. In both experiments sterile distilled water was used to dissolve appropriate quantities of the drugs before it was administered to the animals. The drugs were administered through intramuscular route to animals on the basis of accurate body weight measurement taken immediately before treatment using digital weighing machine (TAL-TEC Livestock Scale, South Africa). All the trypanocidal drugs used for the experiment were tested for their quality (active ingredient) and fulfill certificate of the standards, except for the DA used in the first experiment which had about 70% quality of certification.

4.2.1.6. Feeding and animal management

Animals were fed grass hay and supplemented with concentrates of wheat bran and green Elephant grass. Water and mineral lick were freely available. The handling of animals during the experiment was based on international guiding principles for biomedical research involving animals, as proposed by the Council for International Organizations of Medical Sciences (1985/2012). The research was authorized by the Animal Research Ethics Review Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University (Permit No: VM/ERC/003/04/013).

4.2.2. Parasitological examination, PCV determination and drug resistance tests

Presence of parasites and determination of PCV values were undertaken based on previously established protocols (Murray et al., 1977). Parasitological examination was carried out daily for the first 14 days post infection (dpi), and thereafter twice a week until the end of the experiment. For the drug resistance tests, after all animals of a group became parasitaemic they were treated with the recommended doses (described above) of DA or ISM. From the treatment date cattle were monitored for parasitaemia by the buffy coat technique (Murray et al., 1977) twice a week for 100 days. When relapse (detection of trypanosomes after drug treatment) was detected the animal was treated with a second different drug. If no relapse was detected 100 days after the first and the second trypanocidal drug administration, the treatment was considered successful and the trypanosomes sensitive to drug treatment. Relapse infections detected within 100 days of administration of a trypanocidal drug were taken as indicative of resistance. If relapse occurred in

more than 20% of the cattle tested, the strain was considered resistant to the dose of drug used (Eisler et al., 2001).

4.3. Results

4.3.1. Parasitaemia and PCV values

Clinical forms of trypanosomosis were observed until treatment was applied in all groups of infected animals in both experiments. In the first experiment parasitaemia became patent 5 days post infection (dpi), with the first peak was observed on 7 dpi and the highest peak observed on 14 dpi, when treatment was given in the NT-ETBD1 groups. For the TT-ETBS1 groups parasitaemia became patent later, on 12 dpi, as did the peaks: the first peak was observed on 14 dpi and the highest peak was on 21 dpi, when treatment was given. Correspondingly, in the second experiment establishment of infection was first detected within four days of challenge in animals infected with the non-tsetse isolate while on day seven for tsetse isolate infected groups. All infected animals became parasitaemic and treatment was given on day fourteen when they reached peak parasitaemia. Generally, in both experiments isolates from non-tsetse infested area developed parasitaemia quickly as compared to the isolates from tsetse infested area.

The mean PCV values were significantly different ($P = 0.041$) between pre-infection (34 ±2.3 and 33 ±3.5; NT-ETBD1-DA and NT-ETBD1-ISM, respectively) and post-infection (21 ±2.9 and 23 ±4.19; NT-ETBD1-DA and NT-ETBD1-ISM, respectively) in all groups. Similarly, the mean PCV values were significantly different ($P = 0.009$) between pre-infection (35 ± 4.9 and 34 ±2.14; TT-ETBS1-DA and TT-ETBS1-ISM, respectively) and post-infection (24 ±3.2 and 21 ±5.1; TT-ETBS1-DA and TT-ETBS1-ISM, respectively) in all groups. The mean PCV values remained significantly low after treatment for the NT groups (14 dpi) and for the TT groups (21 dpi) as shown in Figure 4.1A, B. Concomitantly, in the second experiment in all cases there was significant reduction in PCV following infection with *T. vivax* compared with before infection ($P < 0.05$). Treatment of infected groups has resulted in significant improvements in the PCV compared to the values at the day of treatment ($P < 0.05$). However, the improvements were not significant particularly in relapse detected groups (Figure 4.1C). The comparison of mean PCV improvement between groups showed that animals in group NT-ETND2-DA and group NT-ETBD3-DA were found to be the least PCV improvement ($P < 0.001$).

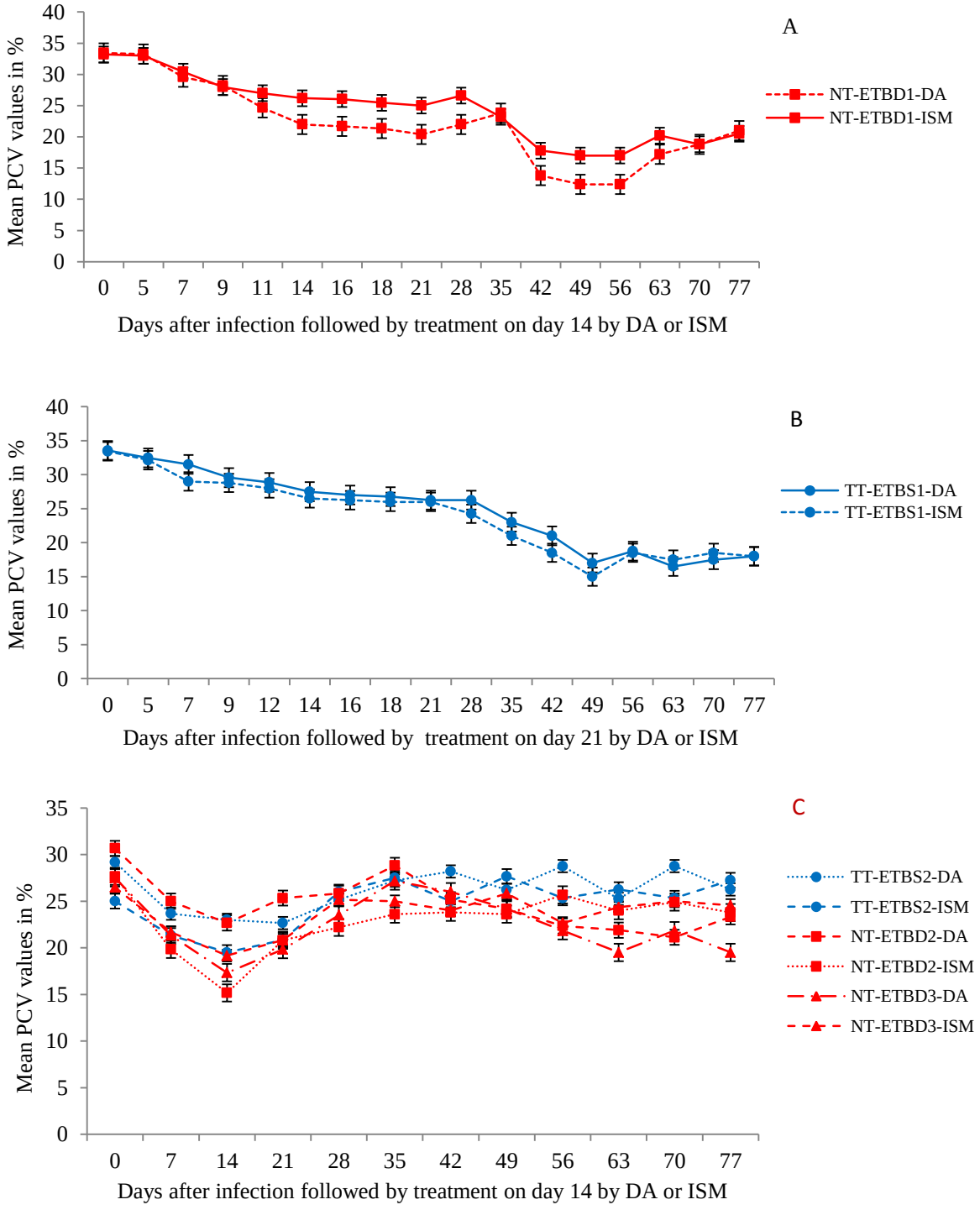
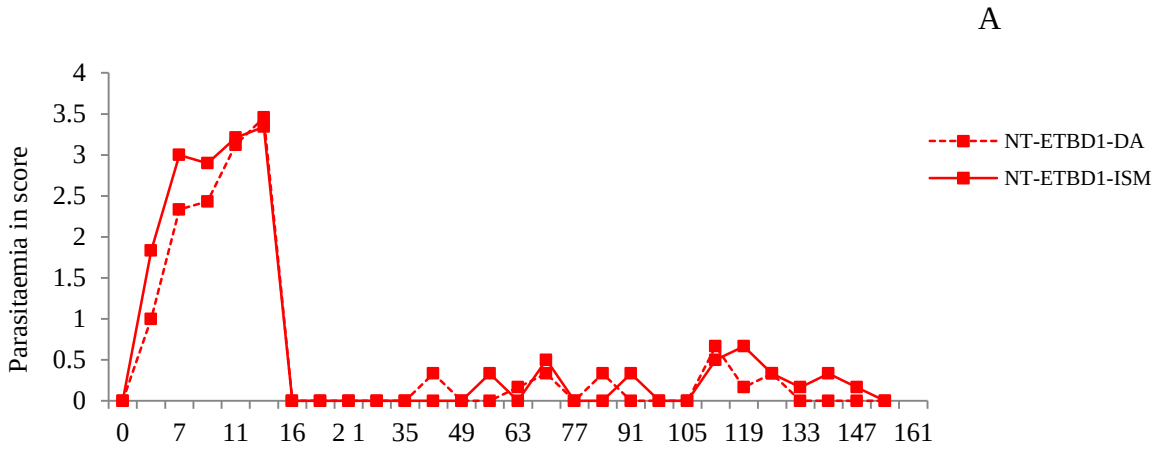


Figure 4.1. Mean ±SE PCV values before and after treatment either by DA or ISM in young Zebu cattle - six animals per group experimentally infected with *Trypanosoma vivax* isolates from tsetse (TT) and non-tsetse infested (NT) areas of Northwest Ethiopia in experiment 1 and 2. (A) NT-ETBD1-DA and NT-ETBD1-ISM, (B) TT-ETBS1-DA and TT-ETBS1-ISM in experiment 1, (C) TT-ETBS2-DA and TT-ETBS2-ISM, NT-ETBD2-DA and NT-ETBD2-ISM, NT-ETBD3-DA and NT-ETBD3-ISM in experiment 2.

4.3.2. Drug resistance test

Prior to treatment, peak parasitaemia was detected in all infected cattle. When the cattle were treated with DA or ISM, the parasitaemia was significantly reduced after 24 hours. However, the assessment of drug resistance tests revealed the occurrence of resistant strains in cattle treated with the recommended doses of both drugs. A total of nine cattle, 4 from the NT groups (2 ISM- treated, and 2 DA- treated) and 5 from the TT groups (3 ISM-treated, and 2 DA- treated), showed detectable relapses of parasitaemia, which began on 35 and 42 days post-treatment (dpt) respectively. The mean wave of parasitaemia in all 6 animals prior to treatment is shown, with the mean of the relapsing 2-3 cattle shown post treatment (Figure 4.2A, B). For the NT groups, relapse of parasitaemia was detected in two animals on 35 and 40 dpt with DA and in another two animals on 37 and 41 dpt with ISM. In the TT groups two animals relapsed 42 and 45 dpt with DA, and three animals on 43, 45 and 56 dpt with ISM. Consequently, more than 20% of the experimental animals in each treatment group demonstrated relapses of parasitaemia indicating the presence of resistant strains against the recommended doses of the trypanocidal drugs tested according to the definitions of Eisler et al. (2001).

In the second experiment a total of five animals showed relapses; three from group NT-ETBD 2-DA) on 21 and 28 dpt, one animal from group NT-ETBD 3-DA) on 35 dpt and another one from group TT-ETBS 2-ISM) on 49 dpt. From the experiment, it was possible to conclude that *T. vivax* isolate (NT-ETBD2-DA) from non-tsetse infested area was found to be resistant at 7 mg/kg body weight of DA. The detection of two relapses one from another non-tsetse infested isolate for DA at 7 mg/kg body weight and one from tsetse infested isolate for ISM at 1 mg/kg body weight is indicative of the presence of resistant strains if more isolates were tested and improved diagnostic tests was used. The mean wave of parasitaemia in all 6 animals prior to treatment is shown, with the mean of the relapsing 1-3 cattle shown post treatment (Figure 4.2C).



Days after infection followed by treatment on day 14 by DA or ISM

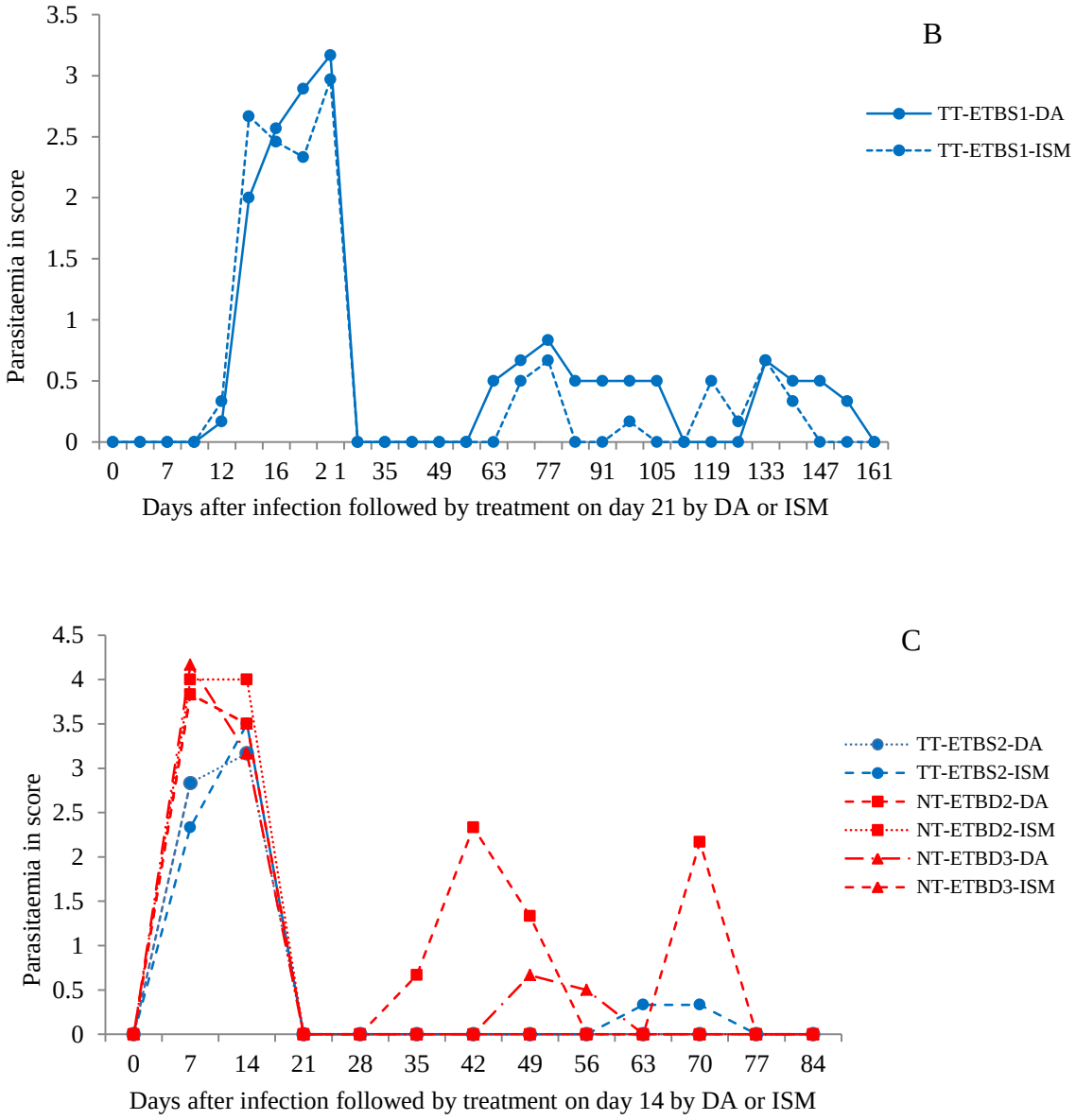


Figure 4.2. Mean parasitaemia in young Zbu cattle - six animals per group experimentally infected with *T. vivax* isolates from tsetse infested (TT-ETBS1 or TT-ETBS2) and non-tsetse infested (NT-ETBD1or ETBD2 or ETBD3) areas followed by treatment either by DA or ISM. It shows the mean of parasitaemia in all 6 animals prior to treatment, but the mean of only the relapsing 1-3 cattle post treatment. (A) From a total of six cattle in NT-ETBD1-DA group two animals showed relapse at 35 and 40 days post-treatment (dpt), and from a total of six cattle in NT-ETBD1-ISM group two animals showed relapse on 37 and 41 dpt, (B) from a total of six cattle in TT-ETBS1-DA group two animals showed relapse on 42 and 45 dpt, and from a total of six cattle in TT-ETBS1-ISM three animals showed relapse on 43, 45 and 56 dpt in experiment 1, (C) from a total of six cattle in each group of NT-ETBD2-DA and NT-ETBD3-DA three and one animals showed relapse at 21 and 28 dpt respectively and from a total six cattle in TT-ETBS2-ISM group one animal showed relapse on 49 dpt.

4.4. Discussion

Trypanosomosis is a major constraint to the utilization of large land resources and also affect livestock, particularly cattle which have a major role in the agricultural economy of Ethiopia (Jemal and Hugh-Johns, 1995). The application of trypanocidal drugs is widely practiced to control trypanosomosis in domestic livestock since several decades either as curative or prophylactic drugs. This long time use of few trypanocides often predisposes the drugs to development of resistance. The questionnaire survey as indicated in the epidemiological investigation of trypanosomosis in Northwest Ethiopia, farmers' practices suggest widespread prevalence of risk factors for emergence of trypanocidal drug resistance. This has prompted us to undertake a controlled experimental trial to investigate the presence of trypanocidal resistance for *T. vivax* originated from both tsetse infested and non-tsetse infested areas of Northwest Ethiopia.

4.4.1. Parasitaemia and PCV findings

An earlier onset of parasitaemia was detected in the NT infected cattle compared to the TT infected cattle in both experiments. The possible reason for early appearance of parasitaemia for the non-tsetse origin *T. vivax* parasite is explained in various sections of this thesis. Furthermore faster relapses of infection were also detected in the non-tsetse *T. vivax* origin. Anaemia associated with trypanosome infections is multifactorial and the relative contribution of each mechanism will differ according to the host-parasite model, the phase of anaemia development and the severity of infection. Rapid PCV recovery after treatment with trypanocidal drugs (Holmes and Jennings, 1976) was not observed in the present work. This might be due to the presence of drug resistant populations of trypanosomes below the limits of our microscopical detection, though emergence of trypanosomes from drug inaccessible sites, nutritional imbalance and/or reduced response of the bone marrow due to exhaustion when the infection runs a chronic course cannot be ruled out (Murray and Dexter, 1988). In the second experiment with higher doses of trypanocidal drugs we have noticed improvement in mean PCV values in non-relapsed groups.

4.4.2. Drug resistance tests

The presence of indiscriminate drug utilization practices and subsequent complaints over the efficacy of the available trypanocidal drugs in the present study arras was supplemented by the presence of resistant strains for both isolates of the drugs tested. In the present study relapses of parasitaemia were detected for both isolates and for both drugs tested. This would indicate a drug resistance status for these two isolates of *T. vivax* against both DA and ISM, contrary to the view expressed by Fairclough (1963), who suggested that it is difficult to induce resistance to isometamidium chloride even by repeated low dosages (0.25-0.5 mg/kg body weight). From animals treated with DA at 3.5 mg/kg and ISM at 0.5 mg/kg body weight, relapse was detected in 33% (4/12) and 41.66% (5/12), respectively. These levels of relapse are in agreement with reports by Leeflang et al. (1976) during their studies of the infectivity of Nigerian isolates of *T. vivax* in Zebu cattle, where infections reappeared after treatment with 3.5 mg/kg diminazene aceturate. Rottcher and Schillinger (1985) also claimed that, in the coast province of Kenya, *T. vivax* isolates

that caused haemorrhagic disease were resistant to diminazene aceturate at 3.5 mg/kg. Rowlands et al. (1993) also reported similar findings on field investigations in Ghibe, reporting trypanosome populations resistant with the dosages of 3.5 mg/kg body weight and 0.5 mg/kg body weight for DA and ISM respectively. Similar findings were reported on the occurrence of drug resistance in goats experimentally infected with *T. vivax* against the appropriate curative doses of DA (3.5 mg/kg body weight) and ISM (0.25 mg/kg body weight) (Desalegn et al., 2010). However, in the same study relapses were not detected in goats treated with DA at a dose of 7 mg/kg body weight and ISM at dose of 0.5 mg/kg body weight. Conversely, in naive Boran (*Bos indicus)* cattle, *T. vivax* and *T. brucei* mixed isolates exhibited low pathogenicity and were sensitive to diminazene aceturate at 3.5 mg/kg body weight and isometamidium chloride at 0.5 mg/kg body weight (Olila et al., 2002). In the same study in goats, 4 out of the 8 goats infected with the same isolates expressed low levels of resistance to the same doses of ISM, whereas all 8 infected goats were sensitive to diminazene aceturate at 3.5 mg/kg body weight. The present finding is also in agreement with field detection of resistance to DA (3.5 mg/kg) and ISM (1 mg/kg) against *T. vivax* in Burkina Faso (Sow et al., 2012; Vitouley et al., 2012). Finally, our findings are also in agreement with earlier studies from northern Ivory Coast in cattle on transit from Mali and Burkina Faso and with high prevalence of *T. vivax* infection (Kupper and Walters, 1983), which showed resistance to doses of 0.5-1 mg/kg isometamidium chloride.

Based on the findings of resistance strains in the first experiment, additional *T. vivax* isolates (TT-ETBS2, NT-ETBD2 and NT-ETBD3) from the two areas were tested using 7 mg/kg DA and 1 mg/kg ISM. Consequently, isolate NT-ETBD2 from non-tsetse infested area was found to be resistant to DA (three out of six animals relapsed). Codjia et al. (1993) reported resistance to the maximum recommended dose of DA (7 mg/kg body weight) for *T. congolense* isolates from cattle bred in the Ghibe valley western Ethiopia. Moreover, in the present study the findings of two relapses, one from NT-ETBD3-DA group from non-tsetse infested area and one from TT-ETBS2-ISM group from tsetse infested area suggests the two isolates are suspected for resistance to the two drugs and hence need further investigation. Sow et al. (2012) clearly showed *T. vivax* isolated from Burkina Faso and tested at a dose of 1 mg/kg body weight ISM was found to be resistant.

As it has already been indicated in the epidemiological study, risk factors for prevalence of drug resistance are widespread in the areas from which the parasites were collected. Higher frequency of treatment, possible under dosage and mishandling especially when the drugs are administered by farmers and possible occurrence of poor quality drugs on the market could be reasons for the development of resistance (Vitouley et al., 2011). On the other hand, the difference in the drug resistance profile between different isolates of the parasite could be explained by the possible variation in exposure of the drugs to such risk factors (Holmes et al., 2004) and parasite variations in exposure to treatment.

The current studies showed that Zebu cattle experimentally infected with *T. vivax* from tsetse and non-tsetse infested areas of Northwest Ethiopia develop clinical trypanosomosis. Treatment of infected cattle with the recommend doses of trypanocides showed incomplete parasite clearance, consistent with the occurrence of resistant strains and adding to growing evidence that such

resistance may be a problem. Furthermore at higher doses, one isolate from non-tsetse area was found to resistant to DA while another one isolate from the same area for the same drug and one isolate from tsetse area to ISM were suspected for resistantce. Animals infected with *T. vivax* isolated from non-tsetse area showed, however, shorter prepatent and relapse periods compared to animals infected with an isolate from tsetse infested area. Therefore, the findings show that drug resistance is a major concern in the study areas and hence, appropriate corrective measures be taken in both tsetse infested and non-tsetse infested areas.

Chapter V:

General Conclusions and Perspectives

Trypanosome infections take a variable courses depending on factors associated with both the host and the parasites, but are characterized in most instances by the intermittent fever, intermittent presence of parasites in the blood causing anaemia, and immunosuppression. Despite its considerable impact on livestock productivity and the economic hardship it causes in several countries, less consideration has been given to animal trypanosomosis. In Ethiopia, trypanosomosis is a major constraint to the utilization of large land resources and also affect livestock, particularly cattle which have a major role in the agricultural economy (Jemal and Hugh-Johns, 1995). The application of trypanocidal drugs is the mainstay of controlling trypanosomosis in domestic livestock since several decades either as curative or prophylactic drugs. The long time use of these few trypanocides often predisposes the drugs to the development of resistance. In the Northwest region of Ethiopia, both tsetse and non-tsetse transmitted trypanosomosis is prevalent, however, attempts have not been done to demonstrate the pathogenic importance and trypanocidal drug resistance status of tsetse and non-tsetse transmitted *T. vivax* parasites.

This PhD study tried to reveal the pathological impacts of *T. vivax* isolated from tsetse and non-tsetse infested areas of Northwest Ethiopia and determine their drug resistance status. Epidemiological, parasitological, clinical, haematological, biochemical, cytokine, gross and histopathological investigation techniques were employed to achieve the objectives. Our epidemiological investigations have shown that trypanosomosis caused by *T. vivax* is prevalent and results in pathological conditions as is reflected by the significant reduction in PCV of infected animals. Furthermore, risk factors for the development of drug resistance are prevalent in both areas. This has initiated us to conduct experimental studies on pathological changes in infected animals and to determine drug resistance status of those *T. vivax* parasites collected from tsetse and non-tsetse infested areas of the Northwest Ethiopia. Consequently, the experimental infections confirmed that clinical trypanosmosis was developed in both experiments across all isolates. Regardless of the similar clinical manifestations, the onset of parasitemia and the appearance of first peak were days earlier in NT *T. vivax* infected groups in both experimental studies. Infection doses are similar for both types of parasites and the experimental animals were grouped randomly; managed similarly and sampling days were identical. Perhaps *T. vivax* isolates from non-tsetse area are better adapted to mechanical transmission which is almost similar to syringe passage as opposed to tsetse transmited trypanosomes which were adapted to cyclical transmission. Furthermore, it is believed that such variation in the onset might be attributed to differences in the behavior of the parasites. Therefore, studies should continue on the genetic characterization of the parasites and transmission routes with more isolates of *T. vivax* from both areas to elucidate this difference.

Anaemia was clearly observed as a typical sign of infection of cattle with both tsetse adapted and non-tsetse transmitted *T. vivax* parasites in the two experimental works. This finding corroborates the reduced PCV reported during field investigation in the naturally trypanosome infected cattle of the region where the isolates were collected. However, the minor differences observed in haematological values between isolates need further investigation with more isolates and in different animal hosts.

Parallel to the clinical manifestations and haematological findings, we observed biochemical changes indicative of pathological and functional disturbances in infected groups of animals. Significant reductions were noticed, following infection, in serum glucose, total cholesterol and albumin whereas change in serum total protein was similar between infected and control groups across both experimental studies. The changes of ALP, AST and ALT were significantly increased in infected groups compared to non-infected groups when the two experiments were taken together. Comparisons made between tsetse transmitted and non-tsetse transmitted *T. vivax* isolates revealed notable differences only in albumin and glucose concentrations whereby albumin level was less and glucose level was higher in NT than in TT groups in the first and second experiemnts respectively, which suggest existence of possible variation in the interaction between these components and the parasites. The extent of changes in serum biochemical parameters may vary with several factors, including the strain of the parasite and variability in susceptibility to infection which consequently influences the nature and severity of the responses (Anosa, 1988). Altogether, except for few cases of differences, infection with *T. vivax* derived from both tsetse and non-tsetse areas initiate similar biochemical changes indicative of significant pathology. Further, works with more characterized strains of *T. vivax* isolated from both areas are vital to understand the biochemical differences that exist in the biochemical metabolism between host and parasite so that metabolic targets can be identified in the parasite to develop chemotherapeutic agents.

The immune cytokine responses showed significant differences between infected and non-infected groups of cattle especially for the TT infected groups in both experiments suggesting a profound immunological response has been mounted. However, the lower responsiveness of the two groups infected with the two non-tsetse isolates in the second experiment may suggest reduced immunogenicity of the parasites. This may have an impact on the resistance of the hosts and development of severe clinical disease. Hence, further study is required to investigate the reason behind such differences by looking for additional cytokine markers. Interesting to note that in the second experiment only major significant differences from the control group were observed after 21 days of experimental infection (Table 5.1); perhaps this is not surprising as the non-infected control group in the second experiment, as opposed to the first experiment, had been inoculated with allogeneic non-infected blood which will of course induce a allogeneic reaction during the first weeks of the experiment. Furthermore, such studies will pave a way of exploring markers for vaccine development that should remain the ultimate goal in the fight against trypanosmosis in the future. Various phenotypic markers of pathogenicity observed during the experimental studies, which indicating the comparison of infected and control groups as well as between infected groups are summarized in Table 5.1.

Table 5.1. Phenotypic markers of pathogenicity in *T. vivax* experimental infections of young Zebu cattle.

Phenotypic markers of pathogenicity		**Experimental groups**						
		Experiment 1			**Experiment 2**			
		TT-ETBS1	NT-ETBD1	NIC	TT-ETBS2	NT-ETBD2	NT-ETBD3	NIC
Parasitaemia prepatent period dpi		12	6	-	6	4	4	-
Haematology	PCV	L*	L*	N	L**	L*	L**	N
	Hgb	-	-	-	L**	L*	L**	N
	RBC	-	-	-	L**	L*	L**	N
	WBC	-	-	-	L*	L*	L*	N
	MCV	-	-	-	H*	H*	H*	N
	MCH	-	-	-	N	N	N	N
	MCHC	-	-	-	N	N	N	N
Biochemical values	Glucose	L*	L*	N	L**	L*	L**	N
	Total cholesterol	L*	L*	N	L*	L*	L*	N
	Albumin	L**	L**	N	L*	L*	L*	N
	Total protein	N	N	N	N	N	N	N
	AST	N	N	N	H**	H**	H*	N
	ALT	N	N	N	H*	H*	H*	N
	ALP	H*	H*	N	N	N	N	N
Cytokine values until 21dpi	IFN-γ	L	M	L	M	M	L	M
	TNF-α	L	M	L	M	M	M	M
	IL-10	M	M	L	M	L	L	M
	IL-12	M*	H*	L	M	M	L	M
Cytokine values after 21dpi	IFN-γ	H*	H*	L	H**	L**	L**	L
	TNF-α	H*	H*	L	H**	L**	L	L
	IL-10	H*	H*	L	H	M	L	L
	IL-12	H*	H*	L	M	L	L	L
Body weight gain		-	-	-	L**	L**	L**	N
Number of animals euthanized		2	2	0	1	1	0	0

TT-ETBS1 and TT-ETBS2- infected groups from tsetse infested area, NT-ETBD1, NT-ETBD2 and NT-ETBD3-infected groups from non-tsetse infested area and NIC-non-infected groups. L: Low, M: Midium, N: Normal, H:High, dpi: days post infection; *: significantly different from NIC groups , **: significant difference between TT- and NT-infected group, -: not done.

The presence of indiscriminate drug utilization practices and subsequent complaints over the efficacy of the available trypanocidal drugs in the present study arras was supplemented by incomplete parasite clearance, consistent with the occurrence of resistant strains and adding to growing evidence that such resistance may be a problem. Higher frequency of treatment, possible under-dosage and mishandling especially when the drugs are administered by farmers and possible occurrence of poor quality drugs on the market could be reasons for the development of resistance. On the other hand, the difference in the drug resistance profile

between different isolates of the parasite at higher doses of the drugs might be explained by the possible variation in exposure of the parasite to such risk factors. Furthermore, animals infected with *T. vivax* isolates from non-tsetse infested area showed, however, shorter prepatent and relapse periods compared with animals infected with an isolate from tsetse infested area. This pursue the need of molecular studies with more isolates from both areas to monitor drug resistance, understand the mechanisms involved for the development of resistance, and the discovery of new drug targets for parasite control.

Generally, *T. vivax* parasites from the two locations is almost equally pathogenic, parasite appearance in blood is faster and immune cytokine responses for the most part are lower in case of NT isolate whereas trypanocidal drug resistance is prevalent in both areas. Based on the above conclusions and perspectives the following recommendations are forwarded:

- Our findings, reminded us that the impact of *T. vivax* should not be neglected in both tsetse and non-tsetse infested areas. This requires close scrutiny on *T. vivax* in the tsetse area during cyclical vector control consequence to the fight against tsetse fly transmitted trypanosomosis using SIT where the problem of *T. vivax* infection will continue by mechanincal transmission, and equal attention in the non-tsetse area in the treatment and control programs.

- Drug resistance was found to be a problem in both tsetse and non-tsetse infested areas. Frequent administration, poor utilization and handling could be sources of the problem. Hence, reversal of the situation should be attempted through various means such as sanative pair treatment, reducing treatment frequencies and using quality tested drugs.

- The innovation and formulation of new trypanocidal should be intensified.

References

Abebe, G., 2005. Trypanosomosis in Ethiopia. Ethiop J Biol Sci 4, 75-121.

Abebe, G., Jobre, Y., 1996. Trypanosomosis: a threat to cattle production in Ethiopia. Revue Med Vet 147, 897-902.

Abebe, G., Shaw, M.K., Eley, R.M., 1993. *Trypanosoma congolense* in the microvasculature of the pituitary gland of experimentally infected Boran cattle (Bos indicus). Vet Pathol 30, 40-409.

Abeer, A.A.E., Shaymaa, I.S., 2011. Clinico-pathological and cytological studies on naturally infected camels and experimentally infected rats with *Trypanosoma evansi*. World Appl Sci J 14, 42-50.

Adamu, S., Ige, A.A., Jatau, I.D., Neils, J.S., Useh, N.M., Bisalla, M., Nok, I.A.J., Esievo, K.A.N., 2008. Changes in the serum profiles of lipids and cholesterol in sheep experimental model of acute African trypanosomosis. Afri J Biotech 7, 2090-2098.

Adamu, S., Fatihu, M.Y., Useh, N.M., Mamman, M., Sekoni, V.O., Esievo, K.A.N., 2007. Sequential testicular and epididymal damage in Zebu bulls experimentally infected with *Trypanosoma vivax*. Vet Parasitol 143, 29-34.

Adeiza, A.A., Maikai, V.A., Lawal, A.I., 2008. Comparative haematological changes in experimentally infected Savannah brown goats with *T. brucei* and *T. vivax*. Afri J Biotech 7, 2295-2298.

Afework, Y., Clausen, P.H., Abebe, G., Tilahun, G., Mehlitz, D., 2000. Multiple drug resistant *Trypanosoma congolense* population in village cattle of Metekel district, Northwest Ethiopia. Acta Trop 76, 231-238.

Ainanshe, O.A., Jennings, F.W., Holmes, P.H., 1992. Isolation of drug-resistant strains of *T. congolense* from the lower Shebelle region of southern Somalia. Trop Anim Health Prod 24, 65-73.

Aksoy, S., 2003. Control of tsetse flies and trypanosomes using molecular genetics. Vet Parasitol 115, 125-145.

Allam, L., Ogwu, D., Agbede, R.I.S., Sackey, A.K.B., 2011. Haematological and serum biochemical changes in gilts experimentally infected with *Trypanosoma brucei*. Vet Arhiv 81, 597-609.

Anosa, V.O., 1988. Haematological and biochemical changes in human and animal trypanosome. Revue Elev Me'd Vet Pays Trop 41, 151-164.

Anosa, V.O., Kaneko, J.J., 1983. Pathogenesis of *T. brucei* infection in deer mice (*Peromyscus maniculatus*), light and electron microscopic studies on erythrocyte pathologic changes and phagocytosis. American J Vet Res 44, 645-651.

Anosa, V.O., Isoun, T.T., 1980. Haematological studies on *T. vivax* infection of goats and splenectomized sheep. J Comp Pathol 90, 153-168.

Archivio, S.D., Cosson, A., Medina, M., Lang, T., Minoprio, P., Goyard, S., 2012. Non-invasive *in vivo* study of the *Trypanosoma vivax* infectious process consolidates the brain commitment in late infections. PLoS Negl Trop Dis 7: 1976.

Awobode, H. O., 2006. The biochemical changes induced by natural human African trypanosome infections. Afri J Biotech 5, 738-742.

Baltz, T., Baltz, D., Giroud, C., Crockett, J., 1985. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei, T. equiperdum, T. evansi, T. rhodesiense and T. gambiense*. The EMBO Journal 4, 1273.

Bancroft, G.J., Sutton, C.J., Morris, A.G., Askonas, B.A., 1983. Production of interferons during experimental African trypanosomiasis. Clin Exp Immunol 52, 135-43.

Barrett, M.P., Burchmore, R.J., Stich, A., Lazzari, J.O., Frasch, A.C., Cazzulo, J.J., Krishna, S., 2004. Future prospects in chemotherapy for trypanosomiasis. In: The trypanosomosis (Maudlin, I., Holmes, P.H. and Miles, M.A., Eds), CABI Publishing, pp. 445-458.

Barry, J.D., McCulloch, R., 2001. Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. Adv Parasitol 49, 1-70.

Batista, J.S., Rodrigues, C.M.F., García, H.A., Bezerra, F.S.B., Olinda, R.G., Teixeira, M.M.G., Blanco, B.S., 2011. Association of *Trypanosoma vivax* in extracellular sites with central nervous system lesions and changes in cerebrospinal fluid in experimentally infected goats. Vet Res 42, 63.

Batista, J.S., Riet-Correa, F., Teixeira, M.M.G., Madruga, C.R., Simoes, S.D.V., Maia, T.F., 2007. Trypanosomiasis by *T. vivax* in cattle in the Brazilian semiarid: Description of an outbreak and lesions in the nervous system. Vet Parasitol 143, 174-181.

Belkaid, Y., Sun, C.M., Bouladoux, N., 2006. Parasites and immunoregulatory T cells. Curr Opin in Immunol 18, 406-412.

Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H., Sawadogo, L., 2002. Pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. Vet Parasitol 108, 1-19.

Berger, B.J., Carter, N.S., Fairlamb, A.H., 1993. Polyamine and Pentamidine Metabolism in African Trypanosomes. Acta Trop 54, 215-224.

Billiau, A, Vandenbroeck, K., 2001. IFN-gamma. In: Oppenheim, J. J, Feldman M, eds. Cytokine Reference. A compendium of cytokines and other mediators of host defense. San Diego: Academic Press 1, 641-688.

Biryomumaish, S., Katumguka-Rwakishaya, E., Rubaire-Akiiki, C.M., 2003. Serum biochemical changes in experimental *T. congolense* and *T. brucei* infection in small East Africa Goats. Vet Archive 73, 167-180.

Bisalla, M., Ibrahim, N.D.G., Lawal, I.A., Esievo, K.A.N., 2007. Serum total protein, albumin and albumin globulin ratio in Yankassa sheep experimentally infected with *T. congolense* and immunomodulated with levamizole. J Protozool Res 17, 39-43.

Blanco, A., Montamat, E.E., 1998. Genetic variation among *T. cruzi.* J Exptl Zool 282, 62-70.

Borst, P., 2002. Antigenic variation and allelic exclusion. Cell 109, 5-8.

Brener, Z., 1979. The parasite: Host-parasite relations: In Z & Z Malik Andrade (eds), Trypanosoma cruzi and Chagas disease. Guanabara Koogan, Rio de Janeiro, p. 1-41.

Broden, A., 1904. Les infections à trypanosomes au Congo chez l'homme et les animaux (communication préliminaire). Bull Soc d'Etudes Colon 11,116-139.

Bruce, D., 1895. Preliminary report on the tsetse fly disease or nagana in Zululand Durban: Bennett and Davis.

Brun, R., Hecker, H., Lun, Z.R., 1998. *T. evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). Vet Parasitol 79, 95-107.

Bush, B.M., 1975. Veterinary laboratory manual. William Heinemann medical books. London, pp. 447.

Cadioli, F.A, Marqus, L.C., Machado, R.Z., Alessi, A.C., Aquino, L.P.C.T., Barnabe, P.A., 2006. Experimental *T. evansi* infection in donkeys: haematological, biochemical and histopathological changes. Med Vet Zootech, 58, 749-756.

Carter, N.S., Berger, B.J., Fairlamb, A.H., 1995. Uptake of diamidine drugs by the P2 nucleoside transporter in melarsen-sensitive and resistant *T. brucei brucei*. J Biol Chem 270, 28153-28157.

Cazzulo, J.J., Stoka, V., Turk, V., 1997. Cruzipain, the major cysteine proteinase from the protozoan parasite *Trypanosoma cruzi*. Biol Chem 378, 1.

Central Statistical Agency (CSA), 2010/2013. Agricultural sample survey. Report on livestock and livestock characteristics. Volume II and IV, Addis Ababa, Ethiopia.

Cheesbrough, M., 1999. Medical laboratory manual for tropical countries. ELBS ed. Pitman Press Ltd. Britain.

Cherenet, T., Sani, R.A., Speybroeck, N., Panandam, J.M., Nadzr, S., Van den Bossche, P., 2006. A comparative longitudinal study of bovine trypanosomosis in tsetse-free and tsetse-infested zones of the Amhara Region, Northwest Ethiopia. Vet Parasitol 140, 251-258.

Chitanga, S., Marcotty, T., Namangala, B., Van den Bossche, P., Van Den Abbeele, J., Delespaux, V., 2011. High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. PLoS Negl Trop Dis 5:1.

Clausen, P.H., Pellmann, C., Scheer, A., Tietjen, U., Schares, G., Bauer, B., Peregrine, A.S., Mehlitz, D., 2000. Application of in vitro methods for the detection of drug resistance in trypanosome field isolates. ICPTV Newsletter 2, 9-12.

Clausen, P.H., Wiemann, A., Patzelt, R., Kakaire, D., Poetzsch, C., Peregrine, A., Mehlitz, D., 1998. Use of a PCR assay for the specific and sensitive detection of Trypanosoma Spp. in naturally infected dairy cattle in peri-urban Kampala, Uganda. Ann NY Acad Sci 849:21-31.

Clery, D.G., Mulcahy, G., 1998. Lymphocyte and cytokine responses of young cattle during primary infection with *Fasciola hepatica*. Res in Vet Sci 65, 169-171.

Codjia, V., Mulatu, W., Majiwa, P.A.O., Leak, S.G.A., Rowlands, G.J., Authie, E., Dieteren, G.D.M., Peregrine, A.S., 1993. Epidemiology of bovine trypanosomosis in the Ghibe Valley Southwest Ethiopia. Occurrence of populations of *Trypanosoma congolense* resistant to diminazene, isometamidium and homidium. Acta Trop 53,151-163.

Coles, E.H., 1986. Veterinary Clinical Pathology.4th ed. W.B.S. Saunder Co. Philadelphia.

Connor, R.J, Van den Bossche, P., 2005. African animal Trypanosomes. In JAW Coetzer, RC Tustin, Infection Diseases of Livestock, 2nd ed., Vol 1, Oxford University Press, Cape Town, p. 251-296.

Cortez, A.P., Rodrigues, A.C., Garcia, H.A., Neves, L., Batista, J.S., 2009. Cathepsin L-like genes of *Trypanosoma vivax* from Africa and South America characterization, relationships and diagnostic implications. Mol Cell Probes 23, 44-51.

Dagnachew, S., Sangwan, A.K., Abebe, G., 2005. Epidemiology of bovine trypanosomosis in the Abay (Blue Nile) basin areas of Northwest Ethiopia. Revue D"Elevage Et DeMed Vet Des Pays Trop 58, 151-157.

Daya, T., Abebe, G., 2008. Seasonal dynamics of tsetse and trypanosomosis in selected sites of Southern Nation, Nationalities and Peoples Regional State, Ethiopia. Ethiop Vet J 12, 77-92.

Dayo, G-K., Bengaly, Z., Messad, S., Bucheton, B., Sidibe, I., Cene, B., Cuny, G., Thevenon, S., 2010. Prevalence and incidence of bovine trypanosomosis in agro-pastoral areas of southwestern Burkina Faso. Res in Vet Sci 88, 470-477.

De Koning, H.P., Anderson, L.F., Stewart, M., Burchmore, R.J., Wallace, L.J., Barrett, M.P., 2004. The trypanocide diminazene aceturate is accumulated predominantly through the TbAT1 purine transporter: additional insight on diamidine resistance in African trypanosomes. Antimicrob Agents Chemother 48, 1515-1519.

Degu, F., Ayalew, B., Tewodros, F. Mersha, C., 2012. Occurrence of bovine trypanosomosis, in the Blue Nile river basin, Northwest Ethiopia. Europ J of Appl Sci 4, 129-135.

Delespaux, V., Geysen, D., Van den Bossche, P., Geerts, S., 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol 24, 236-242.

Delespaux, V., 2004. Improved diagnostic of trypanosome infection and drug resistant *Trypanosoma congolense* in livestock. PhD thesis, Université Libre de Bruxelles. p, 135.

Delespaux, V., Geerts, S., Brandt, J., Elyn, R., Eisler, M.C., 2002. Monitoring the correct use of Isometamidium by farmers and veterinary assistants in Eastern Province of Zambia using the isometamidium-ELISA. Vet Parasitol 110, 117-122.

Desalegn, W.Y., Etsay, K., Getachew, A., 2010. Study on the assessment of drug resistance on *Trypanosoma vivax* in Tselemti district, Tigray, Ethiopia. Ethiop Vet J 14, 15-30.

Desquesnes, M., 2004. Livestock Trypanosomosis and their vectors in Latin America. OIE & CIRAD, Paris, pp.190.

Desquesnes, M., Dia. M.L., 2003. *Trypanosoma vivax*: Mechanical transmission in cattle by one of the most common African tabanids, Atylotus agrestis. Exp Parasitol 103, 35.

Desquesnes, M., McLaughlin, G., Zoungrana, A., Davila, A.M., 2001. Detection and identification of Trypanosoma of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. Int J Parasitol 31, 610-614.

Donelson, J.E., 2003. Antigenic variation and the African trypanosome genome. Acta Trop 85, 391-404.

Duffy, C.W., Morrison, L.J., Black, A., Pinchbeck, G.L., Christley, R.M., Schoenefeld, A., Tait, A.C., Turner, M.R., MacLeod, A., 2009. *Trypanosoma vivax* displays a clonal population structure. Int J Parasitol 39, 1475-1483.

Duggan, A. J., 1977. Bruce and the African trypanosomes. The American J Trop Med and Hyg 26, 1080.

Egbe-Nwiyi, T.N., Olushile, O.M., Mshelbwala, F., 2003. The effects of oral Magnesium chloride supplementation on the pathogenicity of *T. brucei* and *T. congolense* infections in rats. Bioscience Res Com 15, 1-7.

Eisler, M.C., Brandt, J., Bauer, B., Clausen, P.-H., Delespaux, V., Holmes, P.-H., Iemobade, A., Machila, N., Mbywambo, H., McDermott J., Mehlitz, D., Murilla, G., Ndungu, J.M., Peregrine, A.S., Sidibe, I.., Sinyangwe, L., Geerts, S., 2001. Standardized tests in mice and cattle for the detection of drug resistance in tsetse transmitted trypanosomes of African domestic cattle. Vet Parasitol 97, 171-182.

Eisler, M.C., McDermott J., Mdachi, R., Brandt J., Murilla G.A, Sinyangwe L., Mubanga, J., Machila N., Mbody Weightambo, H., Coleman, P.G., Clausen P.-H., Bauer, B., Sidibe, I., Geerts, S., Peregrine, A.S., 2000. Rapid method for the assessment of trypanocidal drug resistance in the field. In: The proceeding of the 9th Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE9). Breckenridge, Colorado, USA, pp. 6-11.

Eisler, M.C., Arowolo, R.O., Gault, E.A., Moloo, S.K., Holmes, P.H., Peregrine, A.S., 1994. Isometamidium concentrations in the sera of Boran cattle: correlation with prophylaxis against tsetse-transmitted *Trypanosoma congolense*. Acta Trop 56, 39-50.

Ekanem, J.T., Yusuf, O.K., 2008. Some biochemical and hematological effects of black seed (*Nigella sativa*) oil on *T. brucei* infected rats. Afri J Biomed Res 11, 79-85.

Eneyew, M., Abebe, G., 1997. Bovine trypanosomosis in South Gondar Administrative Zone bordering Lake Tana (Ethiopia) in the apparent absence of Glossina. J Ethiop Vet Assoc 1, 19-34.

Erkelens, A.M., Dwinger, R.H., Bedane, B., Slingenbergh, J.H.W., Wint, W., 2000. Selection of priority areas for tsetse control in Africa; a decision tool using GIS in Didessa Valley, Ethiopia, as a Pilot Study. In: Animal Trypanosomosis: Diagnosis and Epidemiology. FAO/IAEA Coordinated Research Programme. Vienna, Austria.

Esche, C, Shurin, M.R., Lotze, M.T., 2001. IL-12. In: Oppenheim JJ, Feldman M, eds. Cytokine Reference. A compendium of cytokines and other mediators of host defense. San Diego: Academic Press 1, 187-201.

Esievo, K.A.N., Saror, D.I., 1991. Immunochemistry and immunopathology of animal trypanosomiasis. Vet Bull 61, 765-777.

Ezeokonkwoa, R.C., Ezeha, I.O., Onunkwob, J.I., Onyenwea, I.W., Iheagwama, C.N., Agu, W.E., 2012. Comparative serum biochemical changes in mongrel dogs following single and mixed infections of *Trypanosoma congolense* and *Trypanosoma brucei brucei*. Vet Parasitol 190, 56-61.

Fairclough, R., 1963. A comparison of Isometamidium, Samorin, Berenil and Ethidium bromide under field conditions in Kenya. Vet Rec 75, 855-858.

FAO (Food and Agriculture Organization of the United Nations) 2004. Production year book. FAO, Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations) 2000. Production year book. FAO, Rome, Italy.

Fatihu, M.Y., Adamu, S., Umar, I.A., Ibrahim, N.D.G., Eduvie, L.O., Esievo, K.A.N., 2008. Studies on effects of lactose on experimental *Trypanosoma vivax* infection in Zebu cattle. 1. Plasma kinetics of intravenously administered lactose at onset of infection and pathology. Onderstepoort J Vet Res 75, 163-172.

Faye, D., Fall, A., Leak, S., Losson, B., Geerts, S., 2005. Influence of an experimental *Trypanosoma congolense* infection and plane of nutrition on milk production and some biochemical parameters in West African Dwarf goats. Acta Trop 93, 247-257.

Fikru, R., Hagos, A., Roge, S., Reyna-Bello, A., Gonzatti, M.I., Bekana, M., Goddeeris, B.M., Büscher, P., 2014. A Proline racemase based PCR for Identification of *Trypanosoma vivax* in cattle blood. PLoS ONE, 9, e84819.

Fikru, R., Goddeeris, B.M, Delespaux, V., Moti, Y., Tadesse, A., Bekana, M., Claes, F., De Deken, R., Büscher, P., 2012. Widespread occurrence of *Trypanosoma vivax* in bovines of tsetse as well as non-tsetse-infested regions of Ethiopia: a reason for concern? Vet Parasitol 190, 355-61.

Fiorentino, D.F., Zlotnik, A., Mosmann, T.R., Howard, M., O'Garra, A., 1991. IL-10 inhibits cytokine production by activate macrophages. J Immunol 147, 3815-3822.

Forhead, A.J., 1994. Relationship between plasma insulin and triglyceride concentrations in hyper triglyceridemic donkeys. Rev Vet Sci 56, 389-392.

Franco, J.R., Simarro, P.P., Diarra, A., Jannin, J.G., 2014. Epidemiology of human African trypanosomiasis. Clinical Epidemiol 6, 257-275.

Gardiner, P.R., 1989. Recent studies of the biology of *Trypanosoma vivax*. Adv Parasitol 28, 229-317.

Gazzinelli, R.T., Oswald, I.P., James, S.L., Sher, A., 1992. IL-10 inhibits parasite killing and nitric oxide production by IFN-gamma activated macrophages. J Immunol 148, 1792-1796.

Geerts, S., Holmes, P.H., Eisler, M.C., Diall, O., 2001. African bovine trypanosomosis: the problem of drug resistance. Trends Parasitol 17, 25-28.

Geerts, S., Ndung'u, J.M., Murilla, G., Mbwambo, H., Sinyangwe, L., Machila, N., Delespaux, V., Brandt, J., Peregrine, A.S., McDermott, J.J., Holmes, P.H., Eisler, M.C., 2000. In vivo tests for the detection of resistance to trypanocidal drugs: tests in mice and in ruminants. ICPTV Newsletter 2, 6.

Geerts, S., Holmes, P.H., 1998. Drug management and parasite resistance in bovine trypanosomiasis in Africa. PAAT Technical Sciences Series, No. 1, FAO, Rome, p. 31.

Gilbert, M., Jenner, C., Pender, J., Rogers, D., Slingenbergh, Wint, W., 2001. The development and use of the Programme Against African Trypanosomosis Information System (PAAT-IS). Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV) 3, 10-12.

Gomez-Rodriquez, J., Stijlemans, B., De Muylder, G., 2009. Identification of a parasitic modulatory protein triggering the development of suppressive M1 macrophages during African trypanosomiasis. J Infect Dis 200, 1849-1860.

Gow, A.G., Simpson, J.W., Picozzi, K., 2007. First report of canine Africa trypanosomosis in the UK. J Small Anim Pract 48, 658-661.

Gray, M.A., Kimarua, R.W., Peregrine, A.S., Stevenson, P., 1993. Drug sensitivity screening in vitro of populations of *Trypanosoma congolense* originating from cattle and tsetse flies at Nguruman, Kenya. Acta Trop 55, 1-9.

Guilliams, M., Oldenhove, G., Noel, W., Herin, M., Brys, L., 2007. African trypanosomiasis: naturally occurring regulatory T cells favor trypanotolerance by limiting pathology associated with sustained type 1 inflammation. J Immunol 179, 2748-2757.

Hagos, A., 2010. Equine trypanosomosis in Ethiopia: epidemiology, characterization and control. PhD Thesis, KU Leuven, Belgium.

Hanotte, O., Ronin, Y., Agaba, M., 2003. Mapping of quantitative trait loci controlling trypanotolerance in a cross of tolerant West African N'Dama and susceptible East African Boran cattle. Proc Natl Acad Sci USA 100, 7443-7448.

Herbert, W.J., Lumsden, W.H.R., 1976. *Trypanosoma brucei*: A rapid "matching" method for estimating the host's parasitaemia. Exptl Parasitol 40, 427-431.

Herrera, H.M., Aquino, L.P.C.T., Menezes, R.F., 2002. *Trypanosoma evansi* experimental infection in the South American coati (*Nasuna nasua*); Hematological, biochemical and histopathological changes. Acta Trop 81, 203-210.

Hertz, C.J., Filutowicz, H., Mansfiel, J.M., 1998. Resistance to the African trypanosomes is IFN-gamma dependent. J Immunol 161, 6775-6783.

Hilali, M., Abdel-Gavad, A., Nassar, A., Abdel-Wahab, A., 2006. Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with *Trypanosoma evansi*. Vet Parasitol 139, 237-243.

Hoare, C., 1972. The trypanosomes of mammals, a zoological monograph. Blackwell Scientific Publications, Oxford and Edinburgh, pp.749.

Holmes, P.H., Eisler, M.C., Geerts, S., 2004. Current chemotherapy of animal trypanosomiasis. In: Ian Maudlin, Peter H. Holmes and Michael A. Miles (eds.). The Trypanosomiasis. CABI International. Wallingford, UK. pp. 431-444.

Holmes, P.H., Jennings, F.W., 1976. Pathophysiology of parasitic infection (Ed, E.J.L. Soulsby), Academic Press, New York, pp. 199-210.

Hue, G., Lemsre, J.L., Grard, G., Boutignon, F., Dieu, M.C., Janinin, J., Degand, P., 1990. Serum lipid and lipoprotein abnormalities in human African trypanosomosis. Trans Roy Soc Trop Med Hyg 84, 792-794.

Igbokwe, I.O., 1994. Mechanisms of cellular injury in African trypanosomiasis. Vet Bulletin 64, 611-620.

International Guiding Principles for Biomedical Research Involving Animals, 1985/2012. Guideline developed by the Council for International Organizations of Medical Sciences (CIOMS).

ILRAD, 1982. Annual report of the International Laboratory for Research on Animal Diseases. Nairobi , Kenya.

Jemal, A., Hugh-Johns, M.E., 1995. Association of tsetse control with health and productivity in the Didessa Valley, Western Ethiopia. Prev Med 22, 29-40.

Kadima, K.B., Gyang, E.O., Daniel, Saror, I., Esievo, K.A.N., 2000. Serum biochemical values of *Trypanosoma vivax* infected cattle and the effects of lactose in saline infusion. Vet Arhiv 70, 67-74.

Katunguka-Rwakishaya, E., Murray, M., Holmes, P.H., 1999. The influence of energy intake on some blood biochemical parameters in Scottish Blackface sheep infected with Trypanosoma congolense. Vet Parasitol 84, 1-11.

Katunguka-Rwakishaya, E., 1996. Influence of *T. congolense* infection on some blood inorganic and protein constituents in sheep. Rev Elev Med Vet Pays Trop 49, 311-314.

Katugunka-Rwakishaya, E., Parkins, J.J., Fishwick, G., Murray, M., Holmes, P.H., 1995. The influence of energy intake on the pathophysiology of *Trypanosoma congolense* infection in Scottish Blackface sheep. Vet Parasitol 59, 207-218.

Katunguka-Rwakishaya, E., Murray, M., Holmes, P.H., 1992. The pathophysiology of ovine trypanosomiasis: haematological and blood biochemical changes. Vet Parasitol 45, 17-21.

Kaushik, R.S., Uzonna, J.E., Zhang, Y., Gordon, J.R., Tabel, H., 2000. Innate resistance to experimental african trypanosomiasis: differences in cytokine (TNF-, IL-6, IL-10 AND IL-12) production by bone marrow-derived macrophages from resistant and susceptible mice. Cytokine 12, 1024-1034.

Kemp, S.J., Iraqi, F., Darvasi, A., Soller, M., Teale, A.J., 1997. Localization of genes controlling resistance to trypanosomiasis in mice. Nat Genet 16, 194-196.

Kettle, D.S., 1995. Medical and veterinary entomology, 2nd ed. CADI, Wallingford, pp. 225-227.

Kitani, H., Yagi, Y., Naessens, J., Sekikawa, K., Iraqi, F., 2004.The secretion of acute phase proteins and inflammatory cytokines during *Trypanosoma congolense* infection is not affected by the absence of the TNF-gene. Acta Trop 92, 35-42.

Knoppe, T.N., Bauer, B., McDermott, J., Peregrine, A.S., Mehlitz, D., Clausen, P.H., 2006. Isometamidium sensitivity of *Trypanosoma congolense* stocks from cattle in West Africa tested in mice and the drug incubation infectivity test. Acta Trop 97, 108-116.

Kodelja, V., Mller, C., Politz, O., 1998. Alternative macrophage activation associated CC-chemokine-1, a novel structural homologue of macrophage inflammatory protein-a with a Th2-associated expression pattern .J Immunol 160, 1411-1418.

Kupper, W., Wolters, M., 1983. Observations on drug resistance of *Trypanosoma (Nannomonas) congolense* and *Trypanosoma (Duttonella) vivax* in cattle at a feedlot in the northern Ivory Coast. Trop Med and Parasitol 34, 203-205.

Leak, S.G.A., 1999. Tsetse biology and ecology: Their role in the epidemiology and control of trypanosomosis. CABI Publishing Wallingford, UK. p.155.

Leak, S.G.A., Peregrine, A., Mulatu, W., Rowlands, G.J., d'Ieteren, G., 1996. Use of insecticide-impregnated targets for the control of tsetse flies (*Glossina* species) and trypanosomosis occurring in cattle in an area of South-west Ethiopia with a high prevalence of drug-resistant trypanosomes. Trop Med Int Health 1, 599-609.

Leeflang, P., Buys J., Blotkamp, C., 1676. Studies on *Trypanosoma vivax*; infectivity and serial maintenance of natural bovine isolates in mice. Int J Parasitol 6, 413-417.

Lemecha, H., Mulatu, W., Hussein, I., Rege, E., Tekle, T., Abdicho, S., Ayalew, W., 2006. Response of four indigenous cattle breeds to natural tsetse and trypanosomosis challenge in the Ghibe valley of Ethiopia. Vet Parasitol 141, 165-176.

Logan-Henfrey, L.L., Gardiner, P.R., Mahmoud, M.M., 1992. Parasitic protozoa: In: Kreier JP, Baker JR (Eds). Sandiego Academic Press, pp. 157-275.

Lording, P.M., Friend, S.C.E., 1991. Data analysis guide. Interpretation of laboratory results. Australian Vet Pract 21, 186-195.

Losos, G.J., Ikede, B.O., 1972. A review of pathology of diseases in domestic and laboratory animals caused by *T. congolense, T. vivax, T. rhodesiense and T. gambiense*. Vet Pathol 9, 1-7.

Lukins, A.G., 1999. Epidemiology of non-tsetse transmitted trypanosomiasis *Trypanosoma evansi* in propesctive. ICPTV newsletter 1, 5-8.

Magez, S., Caljon, G., 2011. Mouse models for pathogenic African trypanosomes: unraveling the immunology of host-parasite-vector interactions. Parasite Immunol 33, 423-429.

Magez, S., Caljon, G., Tran, T., Stijlemans, B., Radwanska, M., 2010. Current Status of vaccination against African trypanosomiasis. Parasitology 137, 2017-2027.

Magez, S., Schwegmann, A., Atkinson, R., 2008. The role of B-cells and IgM antibodies in parasitaemia and VSG switching in *Trypanosoma brucei* infected mice. PLoS Pathol 4, e1000122.

Magez, S., Radwanska, M., Drennan, M., 2006. Interferon-gamma and nitric oxide in combination with antibodies are key protective host immune factors during *Trypanosoma congolense* infections. J Infect Dis 193, 1575-1583.

Magona, J.W., Walubengo, J., Origin, J.T., 2008. Acute haemorrhagic syndrome of bovine trypanosomosis in Uganda. Acta Trop 107, 186-191.

Mansfield, J.M., Paulnock, D.M., 2005. Regulation of innate and acquired immunity in African trypanosomiasis. Review article. Blackwell Publishing, Ltd. Parasite Immunol 27, 361-371.

Masake, R.A., Majiwa, P.A.O., Moloo, S.K., Makau, J.M., Njuguna, J.T., 1997. Sensitive and specific detection of *Trypanosoma vivax* using the polymerase chain reaction. Exp Parasitol 85, 193-205.

Masiga, D.K., Smyth, A.J., Hayes, P., Bromidge, T.J., Gibson, W.C., 1992. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. Int J Parasitol 22, 909-918.

Matovu, E., Stewart, M.L., Geiser, F., Brun, R., Mäser, P., Wallace, L.J.M., Burchmore, R.J., Enyaru, J.C.K., Barrett, M.P., Kaminsky, R., Seebeck, T., de Koning, H.P., 2003. Mechanisms of arsenical and diamidine uptake and resistance in *Trypanosoma brucei*. Euk Cell 2, 1003-1008.

Mattioli, R.C., Feldmann, G., Hendrickx, W., Wint, J., Jannin, J., 2004. Tsetse and trypanosomiasis intervention policies supporting sustainable animal agricultural development. Food Agr Environ 2, 310-314.

Maxie, M.G., Losos, G.J., Tabel, H., 1979. Experimental bovine trypanosomiasis (*Trypanosoma vivax* and *T. congolense)*. I. Symptomatology and clinical pathology. Trop Med and Parasitol 30, 274-282.

Mbaya, A., Kumshe, H., Nwosu, C., 2012. The mechanisms of anaemia in trypanosomosis: a review. In: Silverberg, D. (ed.): Anaemia. Shanghai: In Tech. pp. 269-282.

McDermott, J., Woitag, T., Sidibé, I., Bauer, B., Diarra, B., Ouédraogo, D., Kamuanga, M., Peregrine, A., Eislera, C., Zessin, K.H., Dieter Mehlitz, D., Clausen, P-H., 2003. Field studies of drug-resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. Acta Trop 86, 93-103.

McLeod, A., Turner, C.M.R., Tait, A., 1997. Detection of single copy gene sequences from single trypanosomes. Mol Biochem Parasitol 84, 267-270.

Mira, S.F., Ralph, R., 1989. Manual of tropical veterinary parasitology. 1st ed. England C.A.B, pp. 181-260.

Molyneux, D.H., Ashford, R.W., 1983. The biology of Trypanosoma and Leishmania, parasites of man and domestic animals. 4 John Street, London, WCIN 2ET. Pp. 144-160.

` Momen, H., 2001. Some current problems in the systematics of trypanosomatids. Int J Parasitol 31, 640-642.

Morlais, I., Ravel, S., Grebaut, P., Dumas, V., Cuny, G., 2001. New molecular marker for *Trypanosoma (Duttonella) vivax* identification. Acta Trop 80, 207-213.

Morrison, L.J., Marcello, L., McCulloch, R., 2009. Antigenic variation in the African trypanosome: molecular mechanisms and phenotypic complex. Cell Microbiol 11, 1724-1734.

Moti, Y., Fikru, R., Van Den Abbeele, J., Büscher, P., Van den Bossche, P., Duchateau, L., Delespaux, V., 2012. Ghibe river basin in Ethiopia: present situation of trypanocidal drug resistance in *Trypanosoma congolense* using tests in mice and PCR-RFLP. Vet Parasitol 189, 197-203.

Moulton, J.E., 1986. Relapse infection after chemotherapy in goats experimentally infected with *Trypanosoma brucei*: Pathological changes in central nervous system. Vet Pathol 23, 21-28.

Mulugeta, W., Wilkes, J., Mulatu, W., Majiwa, P.A.O., Masake, R., Peregrine, A.S., 1997. Long-term occurrence of *Trypanosoma congolense* resistant to Diminazene, Isometamidium and Homidium in cattle at Ghibe, Ethiopia. Acta Trop 64, 205-217.

Mungube, E. O., Vitouley, H.S., Allegye-Cudjoe, E., Diall, O., Boucoum, Z., Diarra, B., Sanogo, Y., Randolph, T., Bauer, B., Zessin, K-H., Clausen, P-H., 2012. Detection of multiple drug-resistant *Trypanosoma congolense* populations in village cattle of south-east Mali. Parasites & Vect 5,155.

Murray, M., Trail, J.C.M., d'Ieteren, G.D.M., 1990. Trypanotolerance in cattle and prospects for the control of trypanosomiasis by selective breeding. Rev Sci Tech 9,369-386.

Murray, M., Dexter, T.M., 1988. Anaemia in bovine African trypanosomiasis: A review. Acta Trop 45, 389-432.

Murray, M., Morrison, W.I., Whitelaw, D.D., 1988. Host susceptibility to African trypanosomiasis: Trypanotolerance. Adv Parasitol 1, 1-68.

Murray, M., Trail, J.C., Davis, C.E., Black, S.J., 1984. Genetic resistance to African Trypanosomosis. J Infect Dis 149, 311-319.

Murray, M.P.K., Mcintyre, W.I.M., 1977. An improved parasitological technique for the diagnosis of African trypanosomosis. Trans R Soc Trop Med Hyg 71, 325-326.

Mwongela, G.N., Kovatch, R.M., Frazil, M.A., 1981. Acute *Trypanosoma vivax* infection in dairy cattle in Coast Province, Kenya. Trop Anim Health Prod 13, 63-69.

Nadia, M.O., Mohamed, F., Ahmed, H.A., 2012. Haematological profile and parasitological diagnosis of *Trypanosoma vivax* infection in Sudanese Nubian goats. University of Khartoum J Vet Med and Anim Prod 3, 28-45.

Nadia, M.O., Kaila, G.J., Eltahir, H.A., Rahman, A.H.A., 2011. Serum biochemical changes in Nubian Goats, Nilotic Dwarf Goats and Garag Ewes experimentally infected with a mechanically transmitted *Trypanosoma vivax* stock. Intr Jour Biol Chem 5, 136-142.

Naessens, J., Kitani, H., Nakamura, Y., Yagi, Y., Sekikawa, K., Iraqi, F., 2006. Bovine trypanotolerance: a natural ability to prevent severe anaemia and haemophagocytic syndrome? Int J Parasitol 36, 521-528.

Naessens, J., Kitani, H., Nakamura, Y., Yagi, Y., Sekikawa, K., Iraqi, F., 2005. TNF-alpha mediates the development of anaemia in a murine *Trypanosoma brucei* rhodesiense infection, but not the anaemia associated with a murine Trypanosoma congolense infection. Clin Exp Immunol 139, 405-410.

Naisa, B.K., 1967. Follow-up of a survey on the prevalence of homidium resistant strains of trypanosomes in cattle in Northern Nigeria and drug cross-resistance tests on the strains with Samorin and Berenil. Bull Epizoot Dis Afr 15, 231-241.

Nakayima, J., Nakao, R., Alhassan, A., Mahama, C., Afakye, K., 2012. Molecular epidemiological studies on animal trypanosomiasis in Ghana. Parasite &Vect 5, e217.

Namangala, B., De Baetselier, P., Brys, L., 2000. Attenuation of *Trypanosoma brucei* is associated with reduced immunosuppression and concomitant production of Th2 lymphokines. J Infect Dis 181, 1110-1120.

Nantulya, V.M., 1990. Trypanosomiasis in domestic animals: the problems of diagnosis. Rev Sci Tech off Int Epiz 9, 357-367.

Nantulya, V.M., 1986. Immunological approaches to the control of animal trypanosomiasis. Parasitol Today, 2, 168-173.

Ngure, R.M, Ndungu, J.M., Ngotho, J.M., Nancy, M.K., Maathai, R.G., Gateri, L.M., 2008. Biochemical changes in the plasma of vervet monkeys (Chlorocebus aethiops) experimentally infected with *Trypanosoma brucei rhodesiense*. J Cell and Anim Biol 2, 150-157.

Nok, A.J., Balogun, E.O., 2003. A bloodstream *Trypanosoma congolense* sialidase could be involved in anaemia during experimental trypanosomiasis. J Biochem 133, 725-730.

Nyeko, J.H.P., Olemoiyol, O.K., Majiwa, P.A.O., Otieno, L.H., Ociba, P.M., 1990. Characterization of trypanosome isolates from cattle in Uganda using species-specific DNA probes reveals predominance of mixed infections. Insect Sci and Its Application 11, 271-280.

Obaleye, J.A., Akinremi, C.A., Balogun, E.A., Adebayo, J.O., 2007. Toxicological studies and antimicrobial properties of some Iron (III) complexes of Ciprofloxacin. Afri J Biotech 6, 2826-2832.

O'Gorman, G.M., Park, S.D.E., Hill, E.W., Meade, K.G., Mitchell, L.C., Agaba, M., Gibson, J. P., Hanotte, O., Naessens, J., Kemp, S.J., MacHugh, D.E., 2006. Cytokine mRNA profiling of peripheral blood mononuclear cells from trypanotolerant and trypanosusceptible cattle infected with *Trypanosoma congolense*. Physiol Genomics 28, 53-61.

Okech, G., Watson, E.D., Luckins, A.G., Makawiti, D.W., 1996. The effect of experimental infection of Boran cattle in early and mid-pregnancy with *Trypanosoma vivax*. British Vet J 152, 441-451.

Olila, D., McDermott, J.J., Eisler, M.C., Mitema, E.S., Patzelt, R.J., Clausen, P.-H., Poetzsch, C.J., Zessin, K.-H., Mehlitz, D., Peregrine, A.S., 2002. Drug sensitivity of trypanosome populations from cattle in a peri-urban dairy production system in Uganda. Acta Trop 84, 19-30.

Olsson, T., Bakhiet, M., Edlund, C., Höjeberg, B., Van der Meide, P.H., Kristensson, K., 1991. Bidirectional activity signals between Trypanosoma brucei and CD81T cells: a trypanosome-released factor triggers interferon-g production that stimulates parasite growth. Eur J Immunol 21, 2447-2454.

Omotainse S.O., Anosa, V.O., 2009. Comparative histopathology of the lymph nodes, spleen, liver and kidney in experimental ovine trypanosomosis. Onderstepoort J Vet Res 76, 377-383.

Opara, M.N., Fagbemi, B.O., 2010. Patho-physiological Effects of Experimental Trypanosoma congolense and *Trypanosoma vivax* Infections in the Grasscutter (*Thryonomys swinderianus*). Nature and Sci 8, 88-101.

Opperdoes, F.R., Hart, D.T., Baudhuin, P., 1986. Biogenesis of glycosomes (microbodies) in the Trypanosomatdae, *T. brucei*. Europ J Cell Biol 41, 30.

Orhue, N.E.J., Nwanze, E.A.C., Okafor, A., 2005. Serum total protein, albumin and globulin levels in Trypanosoma brucei-infected rabbits: Effect of orally administered Scoparia dulcis. Afr J Biotech 4, 1152-1155.

Osaerio, S., Goossens, B., Jeffcoate, I., Holmes, P., 1998. Effects of *Trypanosoma congolense* and nutritional supplements in Djallonké ewes on live weight during pregnancy, postpartum weight, haematology parameters and lamb performance. Res Vet Sci 65, 65-69.

Osman, M.N., Fadl, M., Rahman, A.H.A., 2012. Haematological profile and parasitological diagnosis of *Trypanosoma vivax* infection in Sudanese Nubian goats. J Vet Med and Anim Prod 3, 28-45

Osman, M.N., Kaila, G.J., Eltahir, H.A., Abdel-Rahman, A.H., 2008. Susceptibility of Sudanese Nubian goats, Nilotic dwarf goats and Garag ewes to experimental infection with a mechanically transmitted *Trypanosoma vivax* stock. Pakistan J Biol. Sci 11, 472-475.

Osorio, A.L., Madruga, C.R., Desquesnes, M., Soares, C.O., Ribeiro, L.R.R., Costa, S.C.G., 2008. *Trypanosoma (Duttonella) vivax*: its biology, epidemiology, pathogenesis, and introduction in the New World - a review. Memórias do Instituto Oswaldo Cruz, Brazil 103, 1-13.

Oyewole, O.I., Malomo, S.O., 2009. Toxicological assessment of oral administration of some antisickling agents in rats. Afri J Bioch Res 3, 024-028.

Padmaja, K., 2012. Haemato-biochemical studies of camels infected with trypanosomiasis. Vet World 5, 356-358.

Paris, J., Murray, M., McOimba, F., 1982. A comparative evaluation of the parasitological techniques currently available for the diagnosis of African animal trypanosomosis in cattle. Acta Trop 39, 307-316.

Pays, E., 2006. The variant surface glycoprotein as a tool for adaptation in African trypanosomes. Microbes Infect 8, 930-937.

Peregrine, A.S., Gray, M.A., Moloo, S.K., 1997. Cross-resistance associated with development of resistance to isometamidium in a clone of *Trypanosoma congolense*. Antimicro Agents Chemother 41, 1604-1606.

Philippe, V., Bernard, B., 2006. Immunology and immunopathology of African trypanosomiasis. Anais da Academia Brasileira de Ciências 78, 645-665.

Riviere, J.E., Popich, M.G., 2009. Veterinary pharmacology and therapeutics. 9th ed. Blackwell, pp. 1174-1175.

Robertson, C.M., Coopersmith, M., 2006. The systemic inflammatory response syndrome. Microbes Infect 8, 1382-1389.

Roeder, P.L., Scott, J.M., Pegram, R.G., 1984. *Trypanosoma vivax* infection of Ethiopian cattle in the apparent absence of tsetse. Trop Anim Health Prod 16, 141-147.

Ross, C.A., Barns A.M., 1996. Alteration to one of three adenosine transporters is associated with resistance to Cymelarsan in *Trypanosoma evansi*. Parasitol Res 82, 183-188.

Rottcher, D., Schillinger, D., 1985. Multiple drug resistance in *T. vivax* in the Tana River District of Kenya. Vet Rec 117, 557-558.

Rowlands, G.J., Leak, S.G., Peregrine, A.S., Nagda, S.M., Mulatu, W., d'Ieteren, G.D., 2001. The incidence of new and the prevalence and persistence of recurrent trypanosome infections in cattle in southwest Ethiopia exposed to a high challenge with drug-resistant parasites. Acta Trop 79, 149-163.

Rowlands, G. J., Mulatu, W., Nagda, S.M., Dolan, R.B. d'Ieteren, G. D. M., 1995. Genetic variation in packed red cell volume and frequency of parasitaemia in East African Zebu cattle exposed to drug resistant trypanosomes. Livestock Prod Sci 43, 75-84.

Rowlands, G.J., Mulatu, W., Authié, E., d'Ieteren, G.D.M., Leak, S.G.A., Nagda, S.M., 1994. Effects of trypanosomiasis on growth and mortality of young East African Zebu cattle exposed to drug-resistant trypanosomes. Prev Vet Med 21, 87-101.

Rowlands, G.J., Mulatu, W., Authie, E., Leak, S.G.A., Peregrine, A.S., 1993. Epidemiology of bovine trypanosomosis in the Ghibe valley, southwest Ethiopia. Acta Trop 53, 135-150.

Schönefeld, A., Röttcher, D., Moloo, S.K., 1987. The sensitivity to trypanocidal drugs of *Trypanosoma vivax* isolated in Kenya and Somalia. Trop Med Parasitol 38, 177-180.

Shaw, A.P.M., 2004. Economics of African trypanosomosis, In: Holmes, P. H. and Miles, M. A. (Eds.), The Trypanosomosis, CABI Publishing, Wallingford, UK, pp. 369-402.

Sher, H., Coffman, F.L., 1992. Regulation of immunity to parasites by T cells and T cell-derived cytokines. Annual Review of Immunol 10, 385-409.

Shi, M., Wei, G., Pan, W., Tabel, H., 2006. Experimental African trypanosomiasis: a subset of pathogenic, IFN-gamma-producing, MHC class II-restricted CD41T cells mediates early mortality in highly susceptible mice. J Immunol 176, 1724-1732.

Shi, M., Pan, W., Tabel, H., 2003. Experimental African trypanosomiasis: IFN-gamma mediates early mortality. Eur J Immunol 33, 108-118.

Shimelis, D., Habtamu, G., Getachew, A., 2011. A cross-sectional study on bovine trypanosomosis in Jawi district of Amhara Region, Northwest Ethiopia. Ethiop Vet J 15, 69-78.

Shimelis, D., Sangwan, A.K., Getachew, A., 2008. Assessment of trypanocidal drug resistance in cattle of the Abay (Blue Nile) basin areas of Northwest Ethiopia. Ethiop Vet J 12, 45-59.

Silva, T.M.F., Olinda, R.G., Rodrigues, C.M.F., Câmara, A.C.L., Lopes, F.C., Coelho, M.F.B., Freitas, R.C.I.A., Teixeira, M.M.G., Batista, J.S., 2013. Pathogenesis of reproductive failure induced by *Trypanosoma vivax* in experimentally infected pregnant ewes. Vet Res 44, 1.

Silva, R.A.M.S., Ramirez, L., Souza, S.S., Ortiz, A.G., Pereira, S.R., Duvila, A.M.R., 1999. Hematology of natural bovine trypanosomosis in the Brazilian Pantanal and Bolivian wetlands. Vet Parasitol 85, 87-93.

Sinshaw, A., Abebe, G., Desquesnes, M., Yoin, W., 2006. Biting flies and *Trypanosoma vivax* infection in three highland district bordering Lake Tana, Ethiopia. Vet Parasitol 142, 35-46.

Slingenbergh, J.H.W., 1992. Tsetse control and agricultural development in Ethiopia. World Anim Rev 70, 30-36.

Solano, P., Jamonneau, V., N'Guessan, P., N'Dri, L., Dje, N.N., Miezan, T.W., Lejon, V., Buscher, P., Garcia, A., 2002. Comparison of different DNA preparation protocols for PCR diagnosis of Human African Trypanosomosis in Cote d'Ivoire. Acta Trop 82, 349-356.

Sones, K.R., Njogu, A.R., Holmes, P.H., 1988. Assessment of sensitivity of *Trypanosoma congolense* to isometamidium chloride: a comparison of tests using cattle and mice. Acta Trop 45, 153-164.

Soulsby, E.J.L., 1982. Helminths, arthropods and protozoa of domesticated animals, 7th ed. Tindall, London, pp.630-645.

Sow, A., Sidibé, I., Bengaly, Z., Marcotty, T., Séré, M., 2012. Field detection of resistance to isometamidium chloride and diminazene aceturate in *Trypanosoma vivax* from the region of the Boucledu Mouhoun in Burkina Faso. Vet Parasitol 187, 105-111.

Spence, P.J., Jarra, W., Lévy, P., Reid, A.J., Chappell, L., Brugat, T., Sanders, M., Berriman, M., Langhorne, J., 2013. Malaria's severity reset by mosquito. Vector transmission regulates immune control of *Plasmodium* virulence. Nature 498, 228-231.

Stephen, L.E., 1986. Trypanosomiasis, a veterinary perspective. Pergamon Press, Oxford, UK, pp.551.

Sternberg, J.M., Rodgers, J., Bradley, B., Maclean, L., Murray, M., Kennedy, P.G., 2005. Meningoencephalitis African trypanosomiasis: Brain IL-10 and IL-6 are associated with protection from neuroinflammatory pathology. J Neuroimmunol 167, 81-90.

Stevens, J.R., Brisse, S., 2004. Systematics of trypanosomes of medical and veterinary importance. The Trypanosomiasis, pp. 1-23.

Steverding, D., 2008. The history of African trypanosomiasis. Review. Parasite & Vect 1, 3.

Stijlemans, B., Vankrunkelsven, A., Brys, L., 2010. Scrutinizing the mechanisms underlying the induction of anaemia of inflammation through GPI-mediated modulation of macrophage activation in a model of African trypanosomiasis. Microbes Infect 12, 389-399.

Sutherland, I.A., Holmes, P.H., 1993. Alterations in drug transport in resistant *T. congolense*. Acta Trop 54, 271-278.

Swallow, B.M., 2002. Impact of trypanosomiasis on agriculture. PAAT Technical and Scientific Series, 2, 35-38.

Tabel, H., Wei, G., Bull, H.J., 2013. Immunosuppression: Cause for Failures of Vaccines against African Trypanosomiases. PLOS Neglected Tropical Diseases 7, e2090.

Tabel, H., Wei, G., Shi, M., 2008. T cells and immunopathogenesis of experimental African trypanosomiasis. Immunol Rev 225, 128-139.

Tabel, H., Kaushik, R.S., Uzonna, J.E., 2000. Susceptibility and resistance to *Trypanosoma congolense* infections. Microbes Infect 2, 1-12.

Taiwo, V.O., Olaniyi, M.O., Ogunsanmi, A.O., 2003. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosoma congolense* and *Trypanosoma brucei* infections in sheep. J Isreal Vet Med 58, 435-443.

Taylor, K., Authie, E.M.L., 2004. Pathogenesis of animal trypanosomosis. The Trypanosomiases, pp. 331-353. CABI publishing, pp. 331-353.

Taylor, K., 1998. Immune responses of cattle to African trypanosomes: protective or pathogenic. Int J Parasitol 28, 219-240.

Taylor, K., Lutje, V., Mertens, B., 1996. Nitric oxide synthesis is depressed in *Bos indicus* cattle infected with *Trypanosoma congolense* and *Trypanosoma vivax* and does not mediate T-cell suppression. American Soc for Microbiol 64, 4115-4122.

Taylor, M.A., Coop, R.L., Wall, R.L., 2007. Veterinary parasitology. 3rd ed. UK: Blackwell publishing, pp. 787-788.

Tewelde, N., Abebe, G., Eisler, M.C., McDermott, J., Greiner, M., Afework, Y., Kyule, M., Munstermann, S., Zessin, K.H., Clausen, P.H., 2004. Application of field methods to assess Isometamidium resistance of trypanosomes in cattle in western Ethiopia. Acta Trop 90, 163-170.

Thrusfield, M., 2007. Sampling. In: Veterinary epidemiology. 4th ed. Blackwell Science Ltd., London, UK, pp. 221-254.

Thumbi, S.M., Junga, J.O., Mosi, R.O., McOdimba, F.A., 2010. Spatial distribution of African animal trypanosomiasis in Suba and Teso districts in Western Kenya. BMC Res Notes 3: 6.

Tizard, I.R., Holmes, W.L., York, D.A., Mellors, A., 1977. The generation and identification of haemolysin of *Trypanosoma congolense*. Experintia, 33, 901-902.

Trail, J.C.M., Wissocq, N., D'Ieteren, G.D.M., Kakiese, O., Murray, M., 1994. Patterns of *Trypanosoma vivax* and *T. congolense* infection differ in young N'Dama cattle and their dams. Vet Parasitol 55, 175-183.

Tuntasuvan, D., Sarataphan, N., Nishikawa, H., 1997. Cerebral trypanosomiasis in native cattle. Vet Parasitol 73, 357-363.

Turner, C.M.R., Aslam, N., Dye, C., 1995. Replication, differentiation, growth and the virulence of *Trypanosoma brucei* infections. Parasitology 11, 289-300.

Uilenberg, G., 1998. A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. FAO, Rome, Italy.

Uzonna, J. E., Kaushik, R.S., Gordon, J. R. and Tabel, H., 1999. Cytokines and antibody responses during *Trypanosoma congolense* infections in two inbred mouse strains that differ in resistance. Parasite Immunol 21, 57-71.

Van den Bossche, P., Doran, M., Connor, R.J., 2000. An analysis of trypanocidal drug use in the Eastern Province of Zambia. Acta Trop 75, 247-258.

Van den Bossche, P., Shumba, W., Makhambera, P., 2000. The distribution and epidemiology of bovine trypanosomosis in Malawi. Vet Parasitol 88,163-176.

Ventura, R.M., Paiva, F., Silva, R.A.M.S., Takeda, G.F., Buck, G.A., 2001. *Trypanosoma vivax*: characterization of the spliced-leader gene of a Brazilian stock and species-specific detection by PCR amplification of an intergenic spacer sequence. Exp Parasitol 99, 37-48.

Vickerman, K., 1985. Developmental cycles and biology of pathogenic trypanosomes. British Med Bulletin, 41, 105.

Vickerman, K., Tetley, L., 1978. Biology and ultra-structure of trypanosomes in relation to pathogenesis. In: Pathogenecity of trypanosomes, Proceedings of a workshop, November 20-23, 1978, Nairobi, Kenya, pp. 23- 31.

Vitouley, H.S., Sidibe, I., Bengaly, Z., Marcotty, T., Van Den Abbeele, J., Delespaux, V., 2012. Is trypanocidal drug resistance a threat for livestock health and production in endemic areas? Food for thoughts from Sahelian goats infected by *Trypanosoma vivax* in Bobo Dioulasso (Burkina Faso). Vet Parasitol 190, 349-354.

Vitouley, H.S., Mungube, E.O., Allegye-Cudjoe, E., Diall, O., Bocoum, Z., 2011. Improved PCR-RFLP for the detection of Diminazene resistance in *Trypanosoma congolense* under field conditions using filter papers for sample storage. PLoS Negl Trop Dis 5(7).

Vreysen, M.J.B., Saleh, K.M., Ali, M.Y., Abdulla, A.M., Zhu, Z.R., Juma, K.G., Dyck, V.A., Msangi, A.R., Mkonyi, P.A., Feldmann, H.U., 2000. *Glossina austeni* (Diptera: Glossinidae) eradicated on the island of Unguja, Zanzibar, using the sterile insect technique. J Econ Entomol 93,123-135.

Wei, G., Tabel, H., 2008. Regulatory T cells prevent control of experimental African trypanosomiasis. J Immunol 180, 2514-2521.

Wellde, B.T., Rearson, M.J., Kovatch, R.M., Chumo, W.T., Wykoff, D.E., 1989. Experimental infection of cattle with *T. brucei rhodesiense*. Ann Trop Med Parasitol 83, 133-134.

Whitelaw, D.D., Gardiner, P.R., Murray, M., 1988. Extravascular foci of *Trypanosoma vivax* in goats: the central nervous system and aqueous humor of the eye as potential sources of relapse infections after chemotherapy. Parasitology 97, 51-61.

Whiteside, E.F., 1962. Interactions between drugs, trypanosomes and cattle in the field. In Goodwin & Nimmo-Smith, eds. Drugs, Parasites and Hosts, pp. 116-141.

Wilkes, J.M., Mulugeta, W., Wells, C., Peregrine, A.S., 1997. Modulation of mitochondrial electrical potential: a candidate mechanism for drug resistance in African trypanosomes. Biochem J 326, 755-761.

Witola, W.H., 2004. RNA-interference silencing of the adenosine transporter-1 gene in *Trypanosoma evansi* confers resistance to diminazene aceturate. Exp Parasitol 107, 47-57.

Witola, W. H., Lovelace, C. E. A., 2001. Demonstration of erythrophagocytosis in *Trypanosoma congolense*-infected goats. Vet Parasitol 96, 115-126.

Woodruff, A.W., Topley, E., Knight, R., Downie, C.G.B., 1972. The anaemia of Kalaazar. British J Haematol 22, 319-329.

Wurocheke, A.U., Anthony, A.E., Obidah, W., 2008. Biochemical effects on the liver and kidney of rats administered aqueous stem bark extract of *Xemenia americana.* Afri J Biotechnol 7, 2777-2780.

Yoshihara, K., Morries, A., Iraqi, F., Naessens, J., 2006. Cytokine mRNA profiles in bovine macrophages stimulated with *T. congolense*. J Vet Med Sci 69, 421- 423.

Zewdu, S., Getachew, T., Hagos, A., 2013. Farmers' perception of impacts of bovine trypanosomosis and tsetse fly in selected districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. Vet Res 9, 214.

Ziemann, H., 1905. Beitrag zur Trypanosomenfrage. Zentralbl Bakteriol Parasitenk Infektionskrankh, Abt I, Orig 38, 307-314.

Publications in International Peer-Reviewed Journals

1. Dagnachew, S., Bezie, M., Terefe, G., Abebe, G., Barry, D.J., Goddeeris, B.M., 2015. Comparative clinico-haematological analysis in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse areas of Northwest Ethiopia. Acta Vet Scand 57, 24.
2. Dagnachew, S., Terefe, G., Abebe, G., Barry, D.J., McCulloch, R., Goddeeris, B.M, 2015. *In vivo* experimental drug resistance study on *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Acta Trop 146, 95-100.
3. Dagnachew, S., Terefe, G., Abebe, G., Barry, D.J., Goddeeris, B.M., 2014. Comparative biochemical changes in young Zebu cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Vet Parasitol 205, 451-459.
4. Kenubih, A., Dagnachew, S., Almaw, G., Abebe, T., Takele, Y., Hailu, A., Lemma, W., 2015. Preliminary survey of domestic animal visceral leishmaniasis and risk factors in north-west Ethiopia. Trop Med Int Helth 20, 205-210.
5. Dagnachew, S., Bezie, M., 2015. Review on *Trypanosoma vivax*. Afri J Basic & Appl Sci 7, 41-64.
6. Bezie, M., Dagnachew, S., Girma, M., Tadesse, D., Tadesse, G., 2014. African trypanosomes: virulence factors, pathogenicity and host responses. J Vet Adv 4, 732-745.
7. Bedada, H., Dagnachew, S., 2012. Study on the prevalence of donkey trypanosomosis in Awi zone Northwest Ethiopia. Ethiop Vet J 16, 65-76.
8. Dagnachew, S., 2011. Epidemiology of Bovine Trypanosomosis in Northwest Ethiopia. LAP LAMBERT Academic Publishing GmbH & Co. KG. ISBN: 978-3-8465-5141-7.
9. Dagnachew, S., Shibeshi, S., 2011. Prevalence and vector distributions of bovine trypanosomosis in control (Sibu Sire) and non-control (Guto Gida) districts bordering upper Anger valley of East Wollega Zone, Western Ethiopia. Ethiop Vet J 15, 77-86.
10. Dagnachew, S., Girma, H., Abebe, G., 2011. A cross-sectional study on bovine trypanosomosis in Jawi district of Amhara Region, Northwest Ethiopia. Ethiop Vet J 15, 69-78.
11. Dagnachew, S., Amamute, A., Temesgen, W., 2011. Epidemiology of gastrointestinal helminthiasis of small ruminants in selected sites of North Gondar zone, Northwest Ethiopia. Ethiop Vet J 15, 57-68.
12. Dagnachew, S., Arun, K. S., Abebe, G., 2008. Assessment of trypanocidal drug resistance in cattle of the Abay (Blue Nile) basin areas of Northwest Ethiopia. Ethiop Vet J 12, 45-59.
13. Dagnachew, S., Sangwan, A.K., Abebe, G., 2005. Epidemiology of bovine trypanosomosis in the Abay (Blue Nile) basin areas of Northwest Ethiopia. Revue Élev Méd Vét Pays Trop 58, 151-157.

Conference presentations

1. Dagnachew, S., Terefe, G., Abebe, G., Barry, D.J., Goddeeris, B.M., 2014. Immune cytokines responses in young Zebu (*Bos indicus*) cattle infected with *Trypanosoma vivax* from tsetse or non-tsetse infested areas of Northwest Ethiopia. Proceedings of Annual Meeting of the Non Tsetse Transmitted Animal Trypanosomoses (NTTAT) Group. May 25, 2014, Paris, France.
2. Dagnachew, S., Barry, D.J., Abebe, G., Terefe, G., Goddeeris, B.M., 2013. *Trypanosoma vivax* in Tsetse and Non-Tsetse Infested Areas of Northwest Ethiopia: determination of pathogenicity and drug sensitivity tests. Proceedings of Annual Meeting of the Non Tsetse Transmitted Animal Trypanosomoses (NTTAT) Group. May 26, 2013, Paris, France.
3. Dagnachew, S., Abebe, G., 2007. Studies on tsetse-transmitted trypanosomosis in new settlement areas of Jawi and Quara districts of Amhara region Northwest Ethiopia. In: Proceedings of International Scientific Council for Trypanosomosis Research and Control (ISCTRC). ISCTRC, 29th Meeting held in Luanda, Angola 2007, Publication NO. 124.
4. Dagnachew, S., 2005. Epidemiology of bovine trypanosomosis in the Abay (Blue Nile) basin areas of Northwest Ethiopia. In: Proceedings of International Scientific Council for Trypanosomosis Research and Control (ISCTRC). ISCTRC, 28th Meeting held in Addis Ababa, Ethiopia 2005, Publication NO. 120.

Thesis

- Epidemiology of bovine trypanosomosis in the Abay basin areas of Northwest Ethiopia. MSc Thesis, AAU, FVM, Debre Zeit, Ethiopia, 2004.

- Incidence rate of major postpartum problems and estimation of uterine involution in Welayta Soddo dairy farms (Jersey, Holstein and Cross Breed Cattle). DVM Thesis, AAU, FVM, Debre Zeit, Ethiopia, June, 1999.

Seminar

- Review on *Trypanosoma vivax:* biology, distribution and impact. Seminar on current parasitology topics in PhD course work. AAU, FVM, Debre Zeit, Ethiopia, 2010.

- Bovine trypanosomosis and drug resistance in tsetse infested areas of Africa. Seminar on current topics on basic sciences in MSc course work. AAU, FVM, Debre Zeit, Ethiopia, 2003.

- The impact of stress on hormonal regulation and immune function in domestic animals. Seminar on current topics in livestock production and development in DVM course work. AAU, FVM, Debre Zeit, Ethiopia, 1998.